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CONTENTS

STUDIES ON THE BIOLOGY OF THE SEA URCHIN

I. Superficial and Histological Gonadal Changes in Gametogenic Process of Two Sea Urchins, *Strongylocentrotus nudus* and *S. intermedius*

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Two sea urchins, *Strongylocentrotus nudus* and *S. intermedius* are recently being caught as animals of economical importance in Hokkaido. According to Kinoshita (1958), their animal has amounted to as much as about 7,000 tons in average from 1953 to 1957.

To know the spawning habits of these animals supplies not only one sort of interesting information on their life-history from the biological viewpoint, but also an important part of the basic information on the sea urchin fishery. Such information includes such items as the gametogenic cycle, spawning season and size at first maturity.

Among the studies dealing with the spawning habits of Echinoidea are those by Miller & Smith (1931), Tennent, Gardiner & Smith (1931), who illustrated the cytology of the egg formation of *Echinometra lucunter*, Moore (1934, 1935a), who reported the profiles of the seasonal variation in the relative gonad volume and gonad maturity of *Echinus esculentus* and *Echinocardium cordatum*, Thorson (1946), who presumed the spawning season for eight Danish sea urchins by the trace of the frequency of appearance of larvae in Danish waters, Lasker & Giese (1954), and Bennett & Giese (1955), who investigated the reproductive cycle of *Strongylocentrotus franciscanus* and *S. purpuratus*. Kawana (1938) gave an outline of various items of information on the gonad development of the common Japanese sea urchin, *Strongylocentrotus pulcherrimus*, Tennent & Ito (1941) presented detailed cytological information on the oogenesis of *Mespilia globulus* with special reference to the morphology of the chromosomes during the course of growth in the primary oocytes and meiotic divisions. However, there are virtually no published observations on the changes occurring in the gonads of the two sea urchins, *S. intermedius* and *S. nudus*, during an entire year nor also many other items of information concerned with their ecology.

The present paper describes mainly how the gametes are formed.

Before proceeding further, the author wishes to express his cordial thanks to Prof. T. Tamura and Asst. Prof. H. Ohmi of Hokkaido University, for their constant guidance and invaluable advices during the course of the work, and also their kindness in revision of the original manuscript. Thanks are offered to Messrs. Y. Ogawa, K. Maruyama and T. Awakura, who aided in collecting the materials used.

Material and Method

Regular and arbitrary samples of sea urchins from the adult population (over 40 mm in test diameter) were obtained at regular intervals from Sinori, Ishiya, Muroran and Setana in southern Hokkaido, within the period from June 1956 to November 1958. The collections were usually made by means of a landing net from a depth of 3–5 m. Samples of smaller urchins (under 30 mm in test diameter) were picked up by hand at times from June 1957 to November 1958, on the rocky shores of Ishiya and Muroran. Figure 1 shows the areas and the localities at which collections were made. Approximately 1,500 urchins were examined in this work.

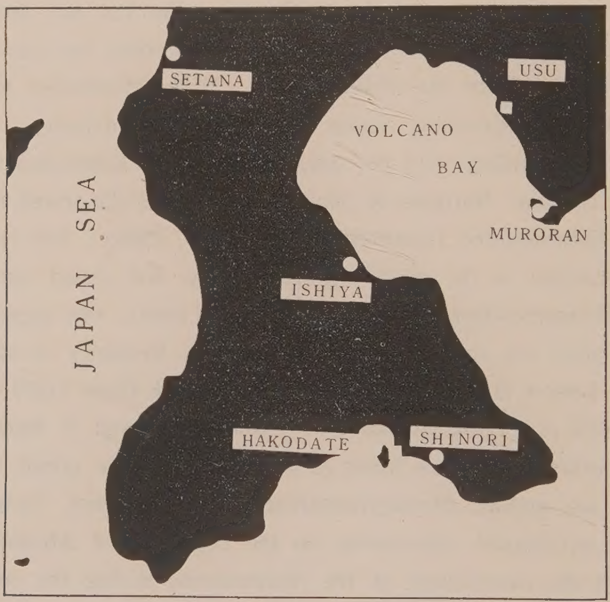


Fig. 1. Sketch-map of southern Hokkaido, showing the localities at which collections were made

Each specimen of sea urchin, after measurement of its test diameter, was immersed into a small vessel (15 × 15 × 10 cm in volume) filled with sea water. The water which overflowed from the vessel was measured as the total volume of the sea urchin. After this treatment, Aristotle's lantern of each specimen was removed, five gonads were removed with care so as to avoid any damage and adhesive water of the gonad surface was blotted with paper. These gonads were examined macroscopically and were then fixed in Bouin's fluid to provide material for histological observation.

There is always some loss of reproductive cells from the cut surface of the gonad, moreover fixation is apt to result in a slight contraction of the gonadal tissue. So all pieces to be fixed were cut large enough to allow for this loss. A small piece was cut off

the central part of the fixed gonad. After normal paraffine embedding, serial sections were prepared between 6μ to 10μ thick, and stained with Delafield's haematoxylin or Heidenhain's iron-alum haematoxylin followed by eosin or light-green as a counterstain.

Observation

General structure of the gonad

The gonads of the Echinoidea are suspended by folds of perivisceral epithelium from the inter-ambulacral plates in the apical half of the body cavity. Each of the gonads possesses a single gonoduct which opens to the exterior through an aperture in the genital plate. The gonoducts are round in cross section, 800 1,000μ in diameter, and they lack any muscle layer, consequently are composed of an inner and outer layer of epithelium with thick connective tissue fibers between.

Observations of the gonad organs show that 13-15 pairs of racemose branches extend to each side of the gonoduct, usually arranged oppositely. In the range up to 20 mm in test diameter all specimens of *S. intermedius* possessed five small gonads which appear semi-transparent to the naked eye. The apices of almost all acini branch in Y shaped form. In the specimens of 20-25 mm test diameter, the aciniformed structure increases in size and dimension; their apices show three or four branches although the superficial feature of their gonads still shows semi-transparency. The ramification of acini of gonads in the animal of over 25 mm test diameter increase greatly in number. Gonads in this stage differ from the former one in their colour; they show yellowish brown. Typical examples of a primary gonad were illustrated in Figure 2. No morphological difference is found in a primary gonad between *S. intermedius* and *S. nudus*. There is no essential difference in gross structure between male and female gonads of the specimens over 40 mm in diameter. Mature ovaries are reddish brown and mature testes are creamy white in colour. On the other hand, when completely contracted or during an early stage of gametogenesis it is extremely difficult to discriminate between the sexes, as all gonads appear reddish brown.

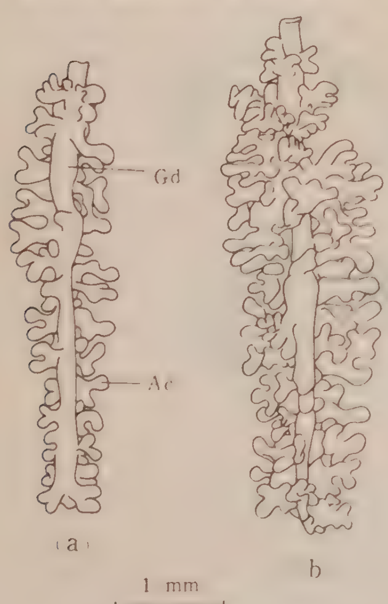


Fig. 2. Primary gonad of *Strongylocentrotus intermedius* to show general morphology Ac., acinus; Gd., gonoduct

The gonad walls of both sexes, in respect to histology, consist of an outer epithelium layer of flat epithelial cells whose distal surface is bathed by perivisceral fluid, a middle layer of conspicuous

definite bands of smooth muscle cells and connective tissue, and an inner layer of developing germ cells. These observations coincide with the findings announced by Wilson (1937, 1940), and by Tennent & Ito (1941) on *Arbacia punctulata* and *Mespilia globulus* respectively.

The male follicle usually contains a few early germ cells near the follicle wall, but the lumen is filled with spermatozoa. The female follicle is occupied by a few young oocytes attached to the wall, while the lumen is packed with large oocytes. Secondary oocytes having a female pronucleus appear polygonal in section, and are packed tightly in the follicles ; the diameter of such oocytes in sections is about 80–100μ.

Gametogenesis in male and female urchin

In describing the gametogenesis in male and female urchin it is convenient to establish a series of arbitrary but easily recognizable stages in its cycle. Six stages including development and regression of gonads are categorized, these being determined in the main by the predominating cell type within a follicle in company with the superficial appearance of the gonads (Fig. 3 and Plates I & II) ; they are defined as follows :

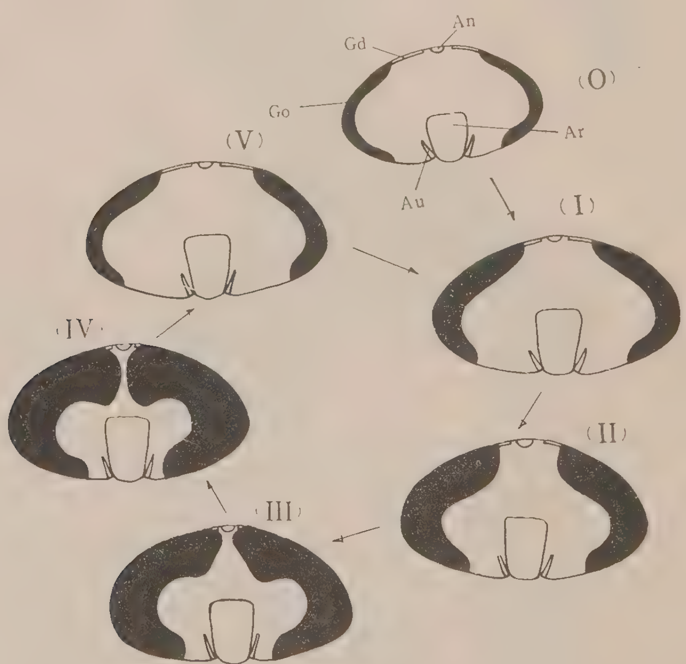


Fig. 3. Diagrammatic figure illustrating cyclic changes in the superficial appearance of the gonad
An., anus; Ar., Aristotle's lantern; Au., auricula; Gd., gonoduct; Go., gonad

Stage 0 (*Neuter*)

S. intermedius of 15–20 mm and *S. nudus* under 30 mm in test diameter possess neuter gonads, which are small, elongated and narrow in dimensions, semi-transparent in

external appearance.

Microscopic examination of sections shows that the lobule-wall of a gonad is very thin. In this stage, therefore, it is impossible to discern any sex difference.

Stage I (*Developing virgin and Recovering spent*)

Growth occurs in dimensions to some extent and sex is recognizable by microscopic examination. *Developing virgin* gonad and *Recovering spent* gonad become very similar to each other in the sectional feature with the exception of occurence of a larger space in the follicle of the latter than of the former.

In macroscopic observation, however, *Developing virgin* gonad differs from *Recovering spent* one in having smaller dimensions. Moreover, *Developing virgin* gonad is still whitish in colour, while *Recovering spent* gonad is reddish brown on account of its developing stage.

Female: Sections of the overy in this stage can be easily recognized, since a very large number of oogonia and a few young oocytes have become attached along the inside of follicle wall. The oogonium is more or less spindle-shaped in form. Its cytoplasm surrounds a prominent nucleus as a thin and homogeneous layer. A young oocyte is irregularly spherical and has one relatively large nucleus surrounded by a thin accumulation of basophilic cytoplasm, which although smooth and homogeneous in apparance is more clearly defined than in the oogonium. The average size of an oocyte is about 15μ in diameter, but some of the youngest ones measure only about 5μ. The nucleus is spherical and sharply limited by its membrane. The nucleolus is situated eccentrically in the reticulum as a chromatin spherule.

Male: The characteristic feature of Stage I testis in section is the presence of numerous spermatogonia and spermatocytes along the follicle wall. It is easily possible to differentiate a spermatocyte from a spermatogonium, as the former is smaller in size and less stainable with haematoxylin than the latter. The spermary of this stage shows low spermatogenic activity. The wall of the male follicle, like the female one, is in a contracted state having many rumples.

Stage II (*Growing*)

Gonads are uniformly reddish brown in colour, showing no differentiation between testis and ovary.

Female: In the periphery of lobules small oogonia are still found, but they are less in number than in the former stage. Various synaptic young oocytes, which connect with one another along the inside of lobules, are arranged in lateral bands. Their diameters now attain 40–60μ. A round germinal vesicle lies in the central region of the oocyte, measuring 20–30μ in size. Further advanced oocytes protrude inward into the follicle adhering to the wall, although they are restricted to a small number. The ovary expands simultaneously with the growth of the oocytes.

Male: The spermary shows vigorous spermatogenesis; production of spermatogonia and spermatocytes advances rapidly along the periphery of the follicles. Consequently, male follicles in section are prominently margined with numerous gametes. No spermatozoa are found in follicles.

Stage III (*Pre-mature*)

Both male and female gonads show a considerable increase in dimensions as compared with Stage II. The sexes are roughly recognizable in this stage by the colour of the gonad; the female gonad is yellowish brown in *S. nudus* and reddish brown in *S. intermedius*, while the male gonad is whitish yellow or creamy white in both species.

Female: A Stage III gonad displays active oogenesis, and is characterized by a great increase in the size of individual advanced oocytes. Numerous large oocytes show an oval appearance, projecting markedly toward the center of the follicle; their dimension is about 80-140μ × 40-80μ. The large germinal vesicle shifts toward the pole of the oocytes and becomes round with a smooth contour. Such ovarian eggs become free from the wall of the follicles with growth, showing spherical or ellipsoidal in shape; their diameter now attains about 80-100μ. They have a large germinal vesicle measuring about 40μ in diameter. Ovarian follicles of this stage possess various oocytes ranging 10-70μ in size, and bear ripe ova in the center of the follicle lumen. In general, however, almost all of the available area in the follicle is occupied by primary oocytes reaching their maximum size.

Male: The spermary in this stage shows very vigorous spermatogenesis as well as in former stage. Spermatocytes and spermatids increase considerably in number; a few spermatozoa migrate centripetally inward from the periphery of the follicle. In more advanced male follicles small sperm patches have been already formed in the center of the follicle, although their area is still limited. Sperm patches are common in Stage III testis; these patches probably consist of the earlier-formed cells.

Stage IV (*Mature*)

Gonads of both sexes reach their peak growth showing maximum dimensions and volume. The gonad acquires a fully matured appearance. The sex of the urchin can be easily distinguished by the colour of the gonads.

Female: Almost available space of the folliclular lumen is packed with circular-shaped secondary oocytes measuring about 80-100μ in diameter, although a very few young oocytes persist near the wall. The mature oocytes in this stage do not differ much from those of the Stage III ovary in size, but their contents show a marked change; the germinal vesicle of these eggs shows signs of breaking down or disappearing, the cytoplasm is homogenous and stains very faintly with haematoxylin.

Male: The spermary is expanded by the ripe spermatozoa, while in the thin membranous wall of the follicle spermatogenesis continues steadily, although the activity slows

down. Almost the entire space of the follicular lumen is fully occupied by mature spermatozoa and shows marked volution in shape owing to aggregation of the spermatozoa.

Stage V (*Spent*)

The spent gonad is thin and of small dimensions in superficial appearance. Both male and female gonads are evenly full whitish-brown in colour. No visible differentiation is notable between an ovary and a testis.

Female: Although microscopic examination of sections shows that the features of the spent gonad vary with time after spawning, in general, the ovary of this stage is characterized by the appearance of an empty space in the center of the follicles, and by the presence of a few unspawned, but apparently ripe, ova. The follicle wall is remarkably shrunk and the middle layer, which consists of muscle fiber and connective tissue, is markedly visible.

The relict ova in the follicle are gradually resorbed by phagocytes, and the gaps of the follicular center are gradually filled with connective tissue. Oogonia and young oocytes increase in number progressively, the ovary again resumes similarity in shape and appearance is that observed in a Stage I gonad.

Male: The most noticeable histological change, additional to the great reduction of spermatozoa, is the presence of gaps in the lumen of the follicle. In some sections a small pactch of relict sperm is observed near the wall or in the lumen.

Relation between gonadal histological stage and gonad weight

The following described experiment was designed to learn how the gonad weight changes with progressive development of gametes and what relationship exists between gonad weight and test diameter or volume of the sea urchin. Figure 4 illustrates the relations between gonad weight and the size or the total volume in the animal belonging to each histological gonad stage as above described. It may be presumed from the above illustration that an exponential relation is found between the gonad weight and the test diameter, while the relationship between test volume and gonad weight is represented by a straight line, within the limit of the data used in this representation.

Correlation coefficient (γ) and correlation ratio (η) are computed from each relation. If the relation between two dimensions (test diameter gonad weight or total volume gonad weight relation), is a completly linear regression, it must be expected that the value of correlation coefficient is equal to the correlation ratio. The significance of the discrepancies between γ and η is tested by the analysis of covariance. Results of analysis obtained are summarized in Table 1. From the above table, it is clearly demonstrated that the relation between the gonad weight and the test volume may be represented by a linear regression extending over all gonad stages, while a linear relation between the gonad weight and the test diameter is calculated in some urchins. Such ratio obtained from smaller individual will be on a lower level than that of larger one. On the other hand,

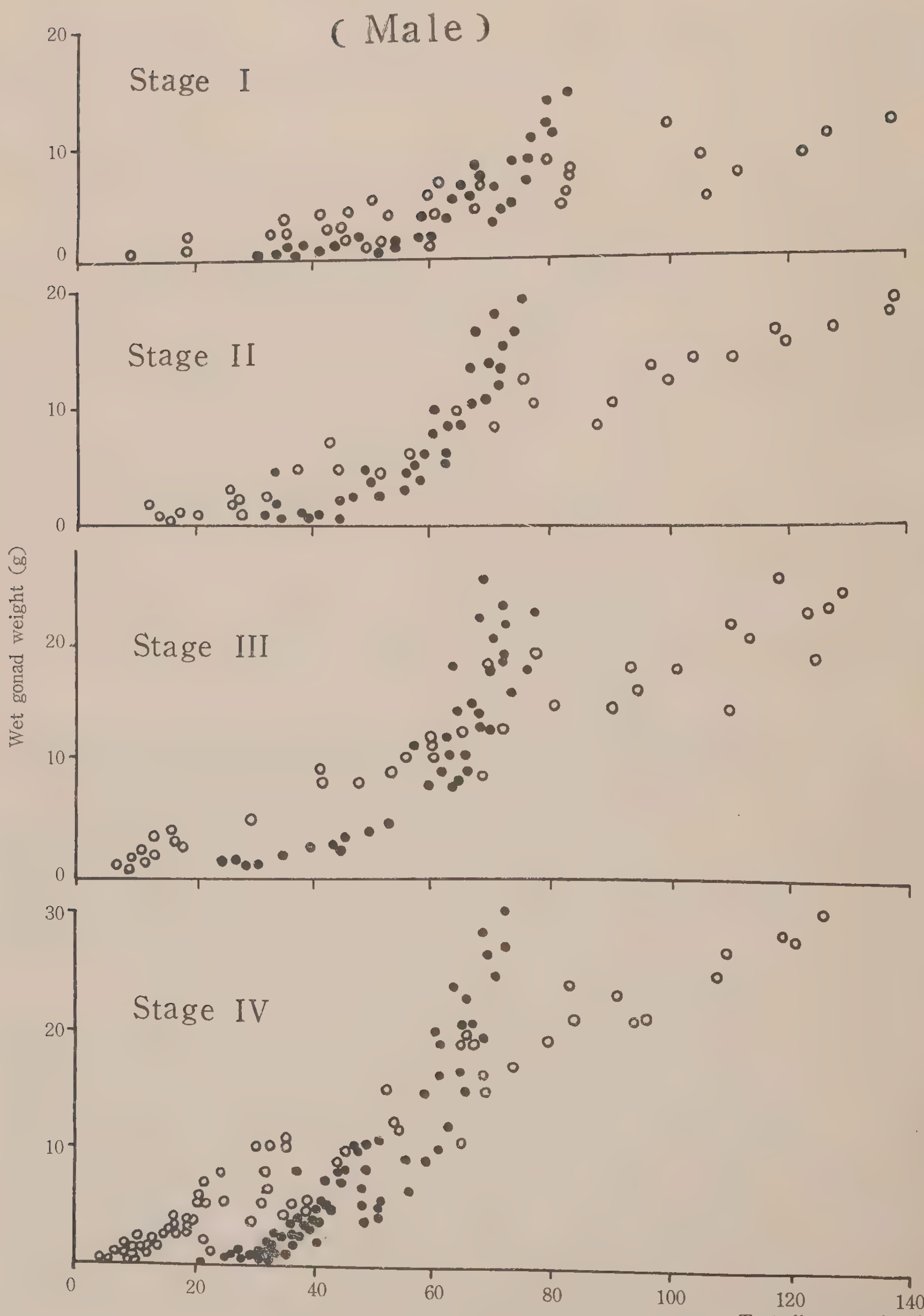
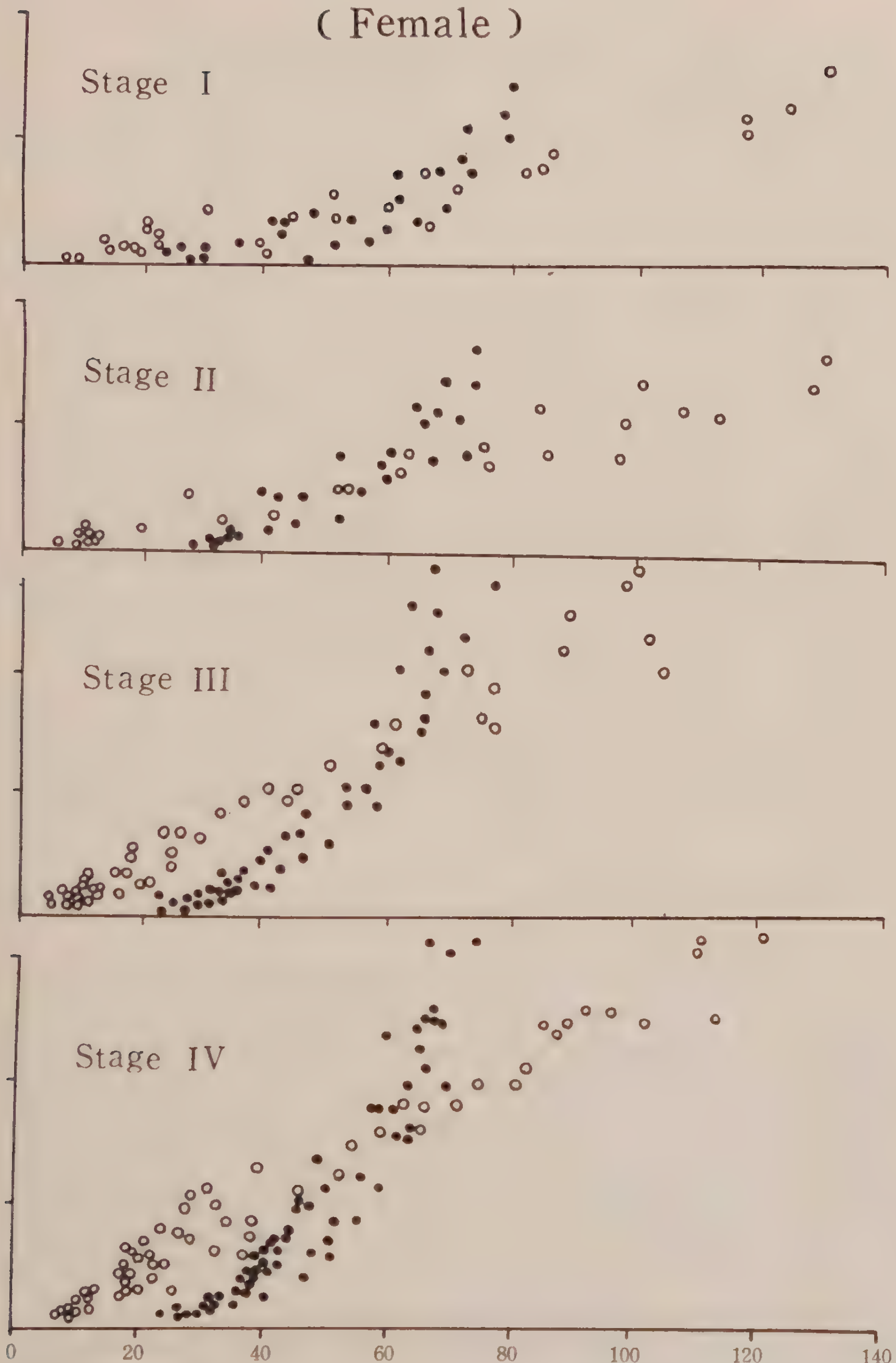


Fig. 4. Scatter diagrams showing the relation between gonad weight
● ; Gonad weight—test diameter relation,



or Total volume (ml)
and test diameter or total volume of *Strongylocentrotus intermedius*
○ ; Gonad weight—total volume relation

Table 1. Test for significance of the discrepancies between γ and η
When the result of the test has significance (Fo>F), linear regression is rejected in the confidence limit of 95 %

a) Wet gonad weight—test diameter relation

Gonad stage	Male			Female		
	γ	η	Fo F	γ	η	Fo F
I	0.693	0.808	2.24<2.64	0.918	0.954	2.59<2.85
II	0.572	0.588	9.09>8.76	0.921	0.976	9.72>2.93
III	0.845	0.899	4.76>2.87	0.901	0.932	4.16>2.62
IV	0.850	0.893	5.27>2.52	0.910	0.932	3.76>2.56

b) Wet gonad weight—total volume relation

Gonad stage	Male γ	Male η	Male Fo F	Female γ	Female η	Female Fo F
I	0.851	0.912	2.24<3.02	0.962	0.984	1.41<2.85
II	0.843	0.867	2.44<3.51	0.976	0.992	1.92<3.13
III	0.906	0.954	1.21<2.63	0.979	0.983	1.43<2.86
IV	0.964	0.959	1.41<2.08	0.955	0.972	1.66<2.67

if the ratio of the gonad weight to the total test volume is computed, it will be found that it shows a constant value irrespectively of the test volume of urchin.

In this paper, *gonad coefficient* is derived from the following formula as a unit of the grade of maturity of a gonad. This is:

$$GC = G_w/V_t \times 100$$

in which GC is the value of gonad coefficient, G_w is wet weight of the gonad in gr and V_t is total test volume of sea urchin in ml.

The gonad cofficient in the samples collected from various locations in southern Hokkaido is compared with each gonadal stage by sex and species (Table 2). Generally, it is assumed from above table that the change in the gonad coefficient with the development of gametes in two sexes runs a slightly different course, though the gonad coefficients of urchins taken at various locations represent a considerable fluctuation even in the creatures of the same gonad stage. In female urchins, a comparatively rapid increase takes place in the gonad coefficients between Stage I and Stage III, and the difference of the gonad coefficients between Stage III and Stage IV has no significance. However, in male gonad, the gonad coefficient shows a gradual augmentation with advance in gonad stages when compared with female urchins.

As already shown in the former section, in female gonads the gametes in the mature ovary do not differ much from these of the pre-mature ovary in size, number and the degree of occupancy within the follicle space, while the female gonads between *Recovering*

Table 2. Comparison of the gonad coefficients in two sea urchins collected from different localities in southern Hokkaido

Gonad stage		Mean gonad coefficient ± standard error						
		S. intermedius				*S. nudus*		
		Muroran	Setana	Ishiya	Shinori	Setana	Ishiya	Shinori
Male	I	10.4±2.1	9.8±2.0	7.0±1.1	7.0±1.0	15.9±2.0	10.6±1.3	11.9±1.1
	II	19.2±1.8	13.1±1.6	13.1±2.0	16.3±2.0	18.6±1.1	12.6±2.1	16.9±2.0
	III	22.6±0.6	16.9±2.7	18.9±2.1	22.0±1.9	23.3±2.8	16.5±2.1	20.1±1.6
	IV	25.7±1.9	20.7±2.0	22.4±3.0	25.1±2.8	25.6±2.0	19.5±1.8	24.9±1.9
	V	7.2±1.1	3.5±1.0	4.2±0.6	6.2±0.9	3.9±0.7	9.7±0.3	6.3±0.9
Female	I	12.2±0.9	10.6±1.0	6.5±0.8	8.7±0.9	14.1±1.9	8.7±0.8	13.3±1.6
	II	20.6±0.8	14.7±2.4	11.8±2.3	17.7±1.8	22.3±2.1	13.0±1.0	18.1±2.1
	III	24.5±2.4	17.8±1.1	21.8±1.2	24.8±2.0	27.1±1.6	18.2±2.6	24.3±2.4
	IV	28.6±1.8	20.7±1.7	25.3±2.9	28.3±2.6	27.9±3.5	24.0±2.1	26.3±2.8
	V	4.8±0.8	4.3±0.6	4.3±0.6	5.3±1.0	5.2±1.2	11.5±2.2	5.7±1.0

spent and *Pre-mature* stage show a considerable difference in size and number of the gametes therein. The male gonad continuously produces germ cells in various developmental stages during the course of advancement in the gonad stages; resultant change is a gradual increment in size and number of the gametes.

Discussion

The most striking feature about the gametogenic cycle of these two urchins is the preponderance of phagocytic cells, stained strongly with haematoxylin, within the follicles after spawning. The resorption of residual germ cells by phagocytosis is well known to occur in bivalve molluscus (Loosanoff, 1937, 1942; Tateishi & Adachi, 1957; Tranter, 1958; Marson, 1958), and such an activity displayed by holothuria phagocytes has already been described by Oshima (1925) and Tanaka (1958). In the present observation, some relict ova showing degeneracy of cytoplasm are noted within the spent follicles; it may be assumed that such indistinct appearance of residual ova are caused by destruction by phagocytic cells.

The question concerned in the origin of germ cells is one of basic and interesting information in respect to the gametogenic process of echinoids. Tennent, Gardiner & Smith (1931) on *Echinometra lucunter* suggested that the cells of the outer epithelium layer of the gonad wall contributed to the visible supply of germ cells by migrating through the

layer of muscle fibers to the inner epithelium. In their paper dealing with the morphology of oogonesis in *Mespilia globulus*, Tennent & Ito (1941) concluded that the outer epithelial layer of the ovary was the true germinal layer supplying the mother cells of the oogonia, which migrate through the muscular layer toward the lumen of the ovary where they divide, differentiate and enlarge. In the present investigation no attempt was made to trace the origin of the germ cells, but it seems that the smallest cells formed on the inner layer of walls resemble closely the cells of the outer epithelial layer.

At the present time a few records of hermaphrodite echinoids are available. From the table by Harvey (1956), the hermaphrodite echinoids are characterized, as a rule, by entire gonads of an individual, testes and ovaries in the same animal. More rarely ovarian and testicular tissue develop side by side in the same gonad, as displayed in the detailed histological descriptions by Moore (1935 b), Harvey (1939), Neefs (1952) and Boolootian & Moore (1956). No hermaphrodic individuals were found among the about 1,500 freshed specimens of the two sea urchins, *S. intermedius* and *S. nudus*.

In general, shedding of gametes within one spawning season may be roughly divided into two types; the first one shows several waves of spawning and the other occurs once during the spawning season. In the former case, various gametogenic processes are observed at the different parts of the gonadal tissue, such as clam, oyster (Loosanoff, 1937, 1942) and pearl oyster (Tateishi & Adachi, 1957 ; Tranter, 1958). In other type, it is observed that the gametes developing in the similar degree are shown by entire gonads of an individual. For testing the effect of partial differences in the degree of development of gametes, histological sections were prepared from the margin and the inside of anterior, middle, and posterior portions of the gonad. In the histological aspects of such preparations, so far as the materials employed in the present investigation are concerned, no significant difference is recognized in any of these portion in the five gonad segments. From this observation, it is strongly suggested that the development of gametes progresses dissimilarly over all the gonads in the same specimen.

It is inferred from Table 2 that the values of the gonad coefficient in the two sea urchins here studied show some considerable differences even in the similar histological stages when compared with one another from specimens collected from various habitats. This fact suggests that there may be a discrepancy between the fecundity of the sea urchins collected from the various localities. It is reasonably presumed that these differences may be due to local variation in the standing crop of algae taken as a food by the urchin, although the cause of such differences is not certain at present.

Summary

1. Observations have been made on some superficial and histological features of the gonads of *Strongylocentrotus nudus* and *S. intermedius*, by examination of samples

from four different localities in southern Hokkaido.

2. The primary gonad of two sea urchins consists of 13 to 15 pairs of racemose branches extended on each side of a single gonoduct, usually arranged oppositely. There is no difference in superficial appearances, such as colouration, gross structure and overall dimensions, between the primary gonads of male and female animals.

3. The development of the gonads is categorized into six arbitrary stages by histological and anatomical examination ; they are defined as follow : Stage 0 (*Neuter*), Stage I (*Developing virgin* and *Recovering spent*), Stage II (*Growing*), Stage III (*Premature*), Stage IV (*Mature*), and Stage V (*Spent*).

4. The gonad coefficient, which is the ratio of the wet weight of gonadal tissue to the total test volume, is proposed as a unit for measurement of the maturity grade of the gonad. This is believed to be one of the most convenient units to show the relative gonad weight. The value of the gonad coefficient is closely correlated with the gametogenic development.

5. It is noticed that the gonad coefficient, even in the same histological stage, shows a considerable difference in specimens from various localities.

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EXPLANATION OF PLATES

PLATE I

Strongylocentrotus intermedius

Photomicrographs of transverse sections through gonads in various stages of maturity. Magnification of the figures is about 60 times.

Fig. 1. Stage 0 gonad (*Neuter*).

Fig. 2. Stage I testis (*Developing virgin*).

Fig. 3. Stage I testis (*Recovering spent*).

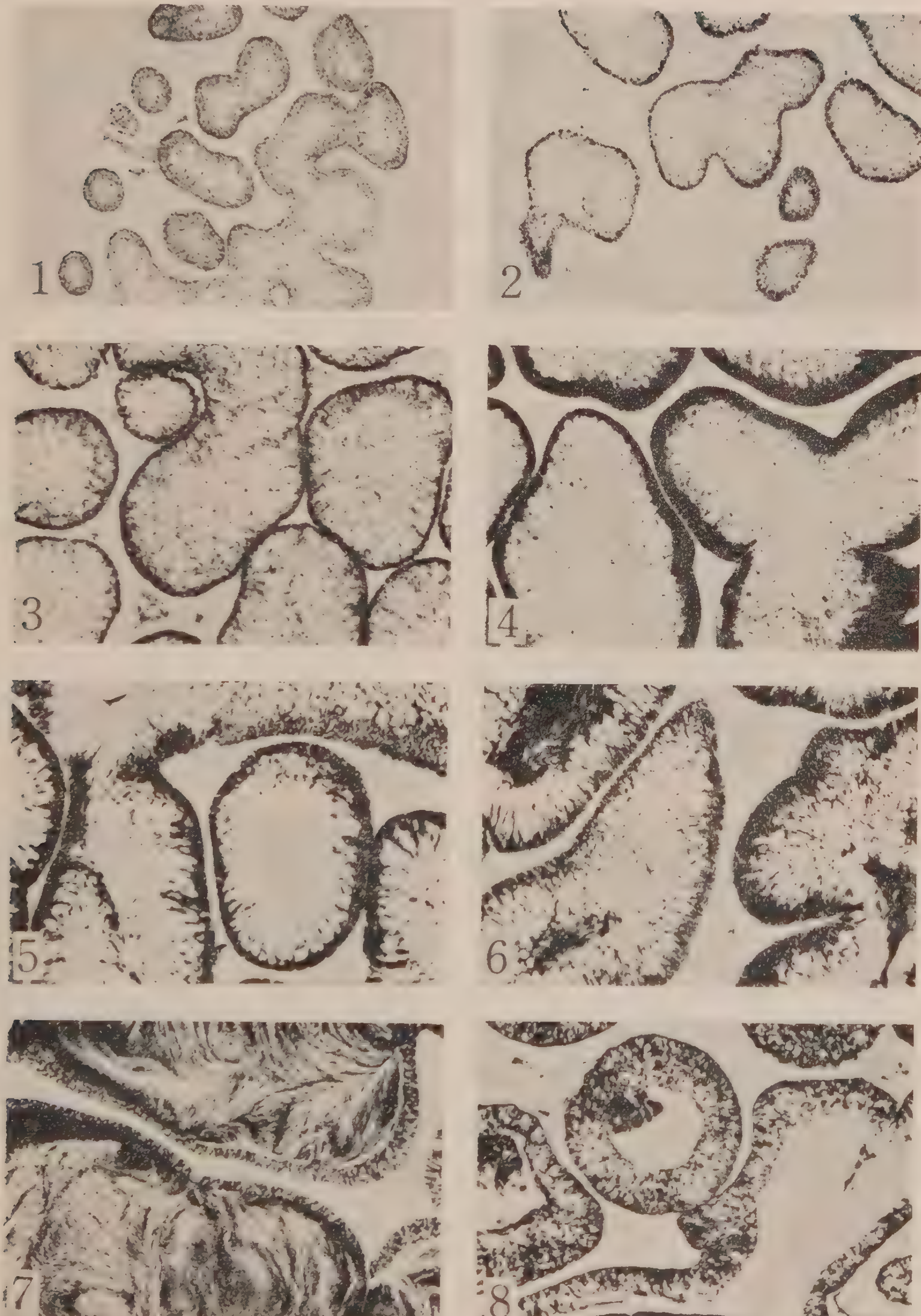
Fig. 4. Stage II testis (*Growing*).

Fig. 5. Stage II testis (*Growing*). Another aspect, showing a slightly advanced testis.

Fig. 6. Stage III testis (*Pre-mature*).

Fig. 7. Stage IV testis (*Mature*).

Fig. 8. Stage V testis (*Spent*). Note small groups of unshedding sperms in lower side.



A. FUJI: Studies on the biology of the sea urchin I

PLATE II

Strongylocentrotus intermedius

Fig. 9. Stage I ovary (*Developing virgin*).

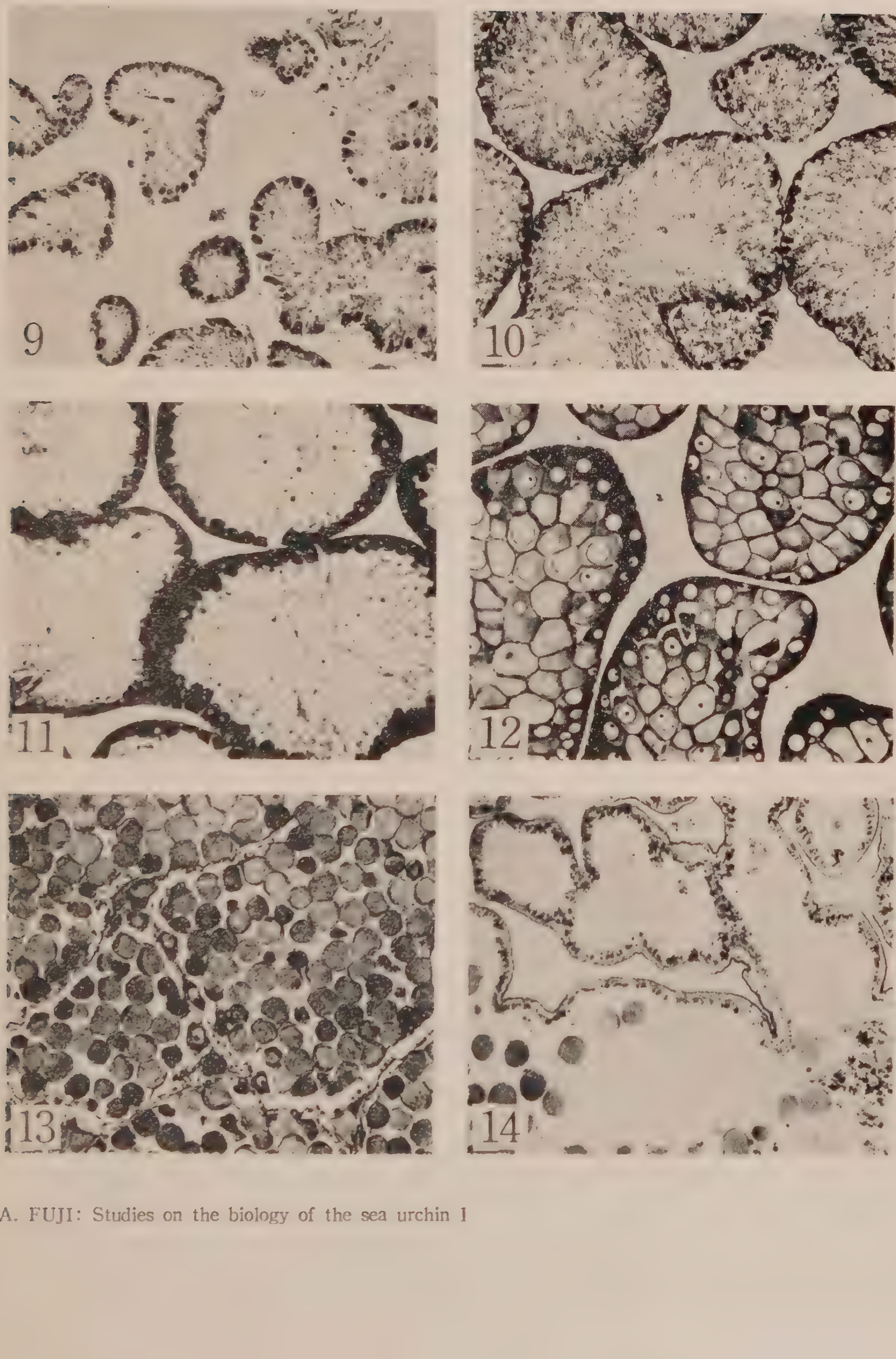
Fig. 10. Stage I ovary (*Recovering spent*).

Fig. 11. Stage II ovary (*Growing*).

Fig. 12. Stage III ovary (*Pre-mature*).

Fig. 13. Stage IV ovary (*Mature*).

Fig. 14. Stage V ovary (*Spent*). Note relict ova in lower side.



A. FUJI: Studies on the biology of the sea urchin I

沿岸洄游時に於けるサクラマスの血液性状の季節的変化
特に滲透圧濃度について

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Notes on the Blood of *Masu* Salmon during Inshore Migration with Special Reference to the Osmoconcentration

Tatsuro KUBO

Abstract

The seasonal changes of osmoconcentration and some other characteristics of blood of *masu* salmon (*Oncorhynchus masou*) migrating in coastal waters of Hokkaido were studied. The osmococentration of blood of young salmon which have been raised once after entrance into the sea in late May tends to decrease shortly afterward. As to the salmon which has passed the winter in the sea, in early spring (January, February), the osmoconcentration of blood is comparatively low, but in late spring (April, May), its significant elevation is found, and it tends to be lowered again, when the salmon approach the estuary. Generally the changes of the water content and the density of blood are seemingly related to the changes in the freezing point of blood, but before the entry into the river, the reduction of the water content and elevation of the density are remarkable conflicting with decreasing of the freezing point.

There may be no direct relationship between these two characteristics and the osmoconcentration of blood. As for the change of the chloride content of blood, the increasing at the first beginning of inshore migration and decreasing before the ascending migration are noticeable.

サケ・マス類の海洋生活時の血液の性状については僅にGreen (1904), Benditt等 (1941), Scholander等 (1957), Gordon (1957) 及び筆者 (1953) の報告があるが，その洄游に関係ある長期間の季節的変化について論じたものは殆んどない。しかしこのような研究はサケ・マス類の洄游移動の要因並びに洄游に伴う魚体内の物質代謝の機構を解明するため，重要である。筆者は以前の報告（久保，1953；1954；1955）でサクラマス (*Oncorhynchus masou*) 幼魚の降海時の血液について述べているが，降海後の海洋生活時のものについての観察を重ねたのでその一部として主に滲透圧濃度に関するものをここに公にする次第である。

材料の採取に際しご北海道大学水産学部小政淳，北海道さけ・ますふ化場小林哲夫，阿部進一，室蘭水族館有馬健二の諸兄より御手伝を戴いた。また魚の捕獲に際しては函館市舘山喜一，岩沢弥太郎，南茅部町浜田常吉，吉田孝夫，木間一雄，松本寅夫，福島町木村徳之助，石狩町中村信一及び室蘭水族館谷口達三の諸氏より多大の配慮を戴いた。ここに記して厚く謝意を表する次第である。

材料及び方法

材料の大部分は第1表中に示される通り，1956年の3月より1960年の2月迄の間，函館を中心とする道南の各地より求められた。河川遡上直前のものとしては石狩川口のものを用いた。但し降海した年の夏，秋のものは天然の材料が得られないため室蘭水族館で飼育したものを用いた。

漁期の初めのもの（第IV群）は全て一本釣によるもので，その他は殆んど定置網によるものである。採血は久保（1953，1954，1955）に示す要領で捕獲直後，漁船上で行い，その分析もまた以前の報告に準じた。

Table 1. Freezing point and some other characteristics of *masu* salmon blood arranged in order of season and date

Phase	Date	Locality	Freezing point (△-C°)	Cl^- content (mg/DL)	Density (Sp. gr.)	Water content (%)	Remarks
I	56- 5-15	Hakodate	0.79	470	1.0618 '523 '506 '504	82.5 83.2 84.2 84.7	smolt
	58- 5-20	Do.	0.84	570	1.0476 '472 '490 '483 '526	85.0 83.7 84.5 85.6	
	57- 5-25	Do.	0.76	460	1.0463 '425 '509 '524 '454 '484	86.2 88.0 83.5	
II	57- 6-10	Muroran	0.73	390	1.0487 '487 '467 '458	83.7 83.3 84.6	reared in aquaria
III	57-10-25	Do.	0.68	425	1.0424 '440 '412	83.0 85.3 82.9	
IV	59- 2- 1	Furube	0.68	430	1.0502	84.0	taken at a depth of over 40m
	Do.	Do.	0.69	410	1.0490	84.2	
	60- 2- 4	Fukushima	0.73	460			
	Do.	Do.	0.69	480			
	Do.	Do.	0.70	460			
	57- 2-23	Nezaki	0.66	405	1.0446	85.5	
	60- 2-25	Furube	0.69	405	1.0518	85.6	
	Do.	Do.	0.68	440	1.0430	85.2	
V	56- 3-29	Nezaki	0.75	370	1.0504	81.5	taken at a depth of about 10 m
	57- 4-13	Hakodate	0.73	390	1.0520	81.8	
	57- 4-23	Do.	0.75	400	1.0504		
	58- 4-25	Do.	0.71	395	1.0500	83.8	
	58- 5- 5	Kotaniishi	0.82	480	1.0572	82.3	
	56- 5-14	Hakodate	0.75	380	1.0579	82.7	
	56- 5-15	Do.	0.73	405	1.0524	82.5	
	57- 5-25	Do.	0.75	415	1.0557	80.2	
VI	58- 5-30	Ishikari	0.71	390	1.0552	80.2	caught in the brackish water of the estuary
	Do.	Do.	0.83	395	1.0554	80.2	
	59- 6- 4	Do.	0.70	365	1.0563	79.4	
	Do.	Do.	0.71	390	1.0563	79.9	
	57- 6- 8	Do.	0.75	375	1.0503	80.3	

なお本文中，冬を過した大型の魚を便宜上成魚と呼ぶこととする。

観 察 の 結 果

Smolt として降海した直後の魚より，河川泝上のため河口部に接近した魚に至る迄，季節並びに月日の順に配列し，その血液の氷点降下度，塩分含量，比重及び水分量を示したものは第1表である。

降海直後と見做されるSmoltは前年に降海した成魚と共に沿岸に洄游するもので，両者全く同一の条件に於て捕獲されるが，Smolt の血液の氷点降下度及び塩分は成魚のそれに比して著しく高い。しかし比較的低い比重と高い水分量という点でSmoltは成魚よりも稀い血液を有する事になろう。

降海直後のSmoltは暫らく沿岸に滞留するが，道南地方では6月以降にはその姿が消え，翌年の1月頃かなり成長した魚となって再び出現するのである。従って実際にこの期間の魚の行動は明かでなく，また血液も入手出来ず，止むを得ず水族館の水槽に飼育された魚について観察した。

勿論水槽に於ては水温，餌料等の条件は天然のそれらと異っている故，魚が正常の状態にあるとは見做し難いが，5月末収容してから相当数のものが元気に生き残つていた所から，それを正常に近いものと仮定し

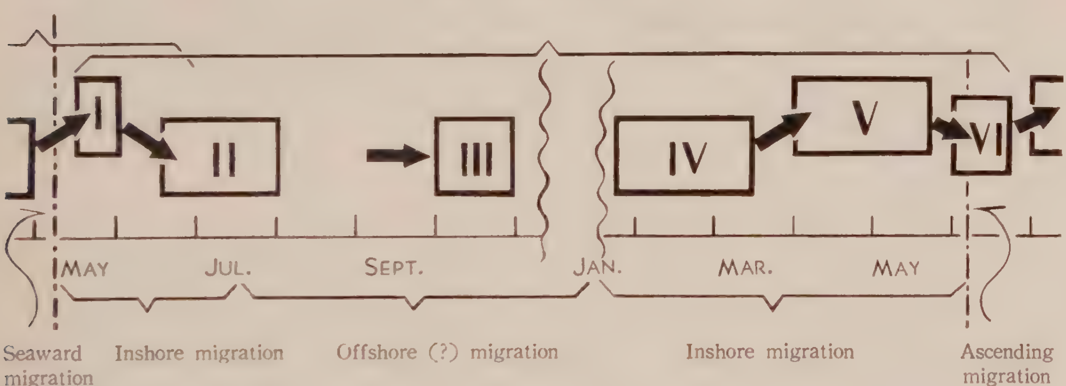


Fig. 1. Diagrammatically represented fluctuation of osmoconcentration of *masu* salmon blood during inshore migration; arrow head symbol shows the gradient of change of concentration between the phases

てその血液の性状を観察した。それによれば降海後一時的に上昇した氷点降下度は △-0.7°C 以下となり，その他の性状に於ても血液は稀められた状態が続くようである。

秋に至ればその氷点降下度は稍上まるが，しかし塩分含量は上昇の傾向を示し，比重及び水分量は大凡以前の状態を保っている。

冬を越して2月に観察された魚の血液は前年秋の魚と相似した状態にあり，塩分含量に於ては高い傾向にあるが，低い氷点降下度，低い比重，高い水分という点から見て，かなり成長し，成熟の進んだ4，5月の魚に比較して血液の濃度は一般的にかなり低い状態にあるものと考えられる。

3月の魚は数が少いため，その状態は明かでないが，4，5月の成魚の血液は前記の2月のものに比べて塩分含量は低減の傾向にあるが，氷点降下度の上昇，比重の増加，水分の減少という点より見て，血液全体の濃縮化即ち滲透圧濃度の上昇がうかがわれる

5月末より6月初めにかけて河口部に接近した成魚の血液は氷点降下度，塩分共僅かに低減するが，比重が以前よりも上昇し，また水分量の減少の著しい事は血液が更に濃化しつつある事を示すようである。

考　　察

第I表に示された魚の血液の性状は各季節毎にかなり明白な特徴を示している。今便宜上それぞれの季節の魚に，魚体の大小，血液の性状並びにその生息環境の差から相の区分を与えるならば，大凡六つの相が想定される。仮にこれらの各相に第1表の左側に区分した通りI～VIの名称を付し，それらの間の変動差異の全般的な比較を試みよう。

血液の諸性状の中で最も重要なものと考えられる氷点降下度の季節的変動の模式的表現を第1図に示した。第Iの相に於ける高い濃度と第IVの相より第Vの相に移る際の上向の傾度とは特に明かである。サクラマスの降海直後の血液は氷点降下度及び塩分含量に於て上昇し，また著しい低比重，高水分の状態を示すが，時間の経過と共に氷点降下度，塩分含量は低減し，比重，水分量も安定の状態に入ることは筆者（未発表）が実験的に Smolt の海水転移を行つて推測している。

第II及びIIIの相に関する天然の資料は将来も入手し難いものであろう。

第IVの相即ち早春に於ける低い氷点降下度はこれ迄の研究者による他のサケマス類の場合には全く例を見なかつた特徴であ。塩分含量は見かけの上でも第IVの相は第Vの相よりも高いが，これを Mol 濃度に換算して塩分の受け持つ氷点降下度の分圧を求めるならば下記の通りとなり，第IVの相の塩分による滲透圧濃度は他の相に於けるよりも高いことが判る。

Phase IV		Phase V		Phase VI	
Total blood F. P. (△−°C)	Equivalent Cl^- F. P. (△−°C)	Total blood F. P. (△−°C)	Equivalent Cl^- F. P. (△−°C)	Total blood F. P. (△−°C)	Equivalent Cl^- F. P. (△−°C)
0.68	0.23	0.75	0.19	0.71	0.20
0.69	0.21	0.73	0.20	0.83	0.21
0.73	0.24	0.75	0.21	0.70	0.19
0.69	0.25	0.71	0.21	0.71	0.20
0.70	0.24	0.82	0.25	0.75	0.20
0.66	0.21	0.75	0.20		
0.68	0.21	0.73	0.21		
0.69	0.23	0.75	0.22		

但し分子凍点降下を1.86°Cとして計算した。

即ち第V及び第VIの相に於ける高い全血液の氷点降下度と比較的低い塩分による氷点降下度は一見矛盾した感じを与える。しかしこの事は第V及び第VIの相の血液の滲透圧濃度に於ては第IVの相と比べて塩分以外のものが受け持つ部分がより大きいであろう事を暗示している。恐らくこの塩分以外のものの主なものは窒素化合物であろうと考えられる。Scholander等（1957）も *Salvelinus alpinus* の血液に於ける氷点降下度と塩分含量の無関係を述べている。見かけの上の塩分含量の変動と相伴わない氷点降下度の変化は魚の生活環境及び活動性と深い関係のある魚体内の窒素代謝の変化に影響されているかも知れない。実際に第IVの相の魚は一般に 40m 位の深層に生息し，水温の冷たい（2～4°C）上層には現れない。しかし季節の推移と共に魚は漸次上層に浮上し泝河前の第VIの相の魚は殆んど表層（水温12°C以上）を游泳するに至る。これと共に魚の活動性も大となる。この事は漁獲方法の差異としても現れ，第IVの相の魚は釣によつて獲られるが，第V及びVIの相の魚は建網で獲られるのが普通である。

環境及び習性の変化は血液の比重と水分量にも反映している。即ち比重は第IVよりVIの相に向つて次第に高まり，逆に水分量は低くなり，血液は一種の濃化を起す傾向にあるが，これは筆者の資料（未発表）によれば蛋白質の濃度と関係があるようである。黒田（1941）は，淡水海水両方に生活出来る魚（ウナギ，ボラ，スズキ）では海水生活時には血液水分量が大であり淡水生活時にはそれが小となる事を述べ，また活動性の大きい魚の血液水分量は小さい魚のそれよりも小である事を明かにしている。この原則は本研究におけるサクラマスの場合にもよく当てはまる事になる。

第VIの相における氷点降下度と塩分含量の低減の傾向は，恐らく河口部の淡水に接したため，漸次滲透圧濃度が低下しつつあるものらしく，Green（1904）のマスノスケ，Benditt等（1941）の Atlantic salmon の場合と似ているが，しかし実際にその後の時期の血液の滲透圧濃度は再び上昇する可能性がある（久保，未発表）ので，溯河と共に滲透圧濃度は単純な過程を経て低減するものとは断定し難い。

摘　　要

沿岸洄游時のサクラマスの血液の性状の季節的変化を調べた。

氷点降下度は降海後一時上昇するがその後低減するものらしく，翌年の1，2月の低い値と4，5月の高い値，及び溯河前の低減が目立つている。

塩分含量も降海後の一時的な上昇が見られ，翌年4，5月より溯河時にわたつて低減が起る。しかしその変動は必ずしも氷点降下度のそれとは一致しない。

比重は降海の翌年4，5月に上昇を始め，また水分量は同じく4，5月に低減を始め，特に溯河前それが著しい。

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NEW TYPE OTTER-BOARDS FOR MID-WATER TRAWL

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It is well-known that the wider the mouth of a trawl net opens, the better the catch of fish. But the mouth of the trawl net is apt to be shut by fluid resistance of the net while being towed. A pair of otter-boards is the only device which has been employed up to now to make the mouth of the trawl net open horizontally, though many inventions have succeeded to make it open vertically. Various devices and contrivances on otter-boards themselves, especially on otter-boards for a mid-water trawl, have been brought into use.

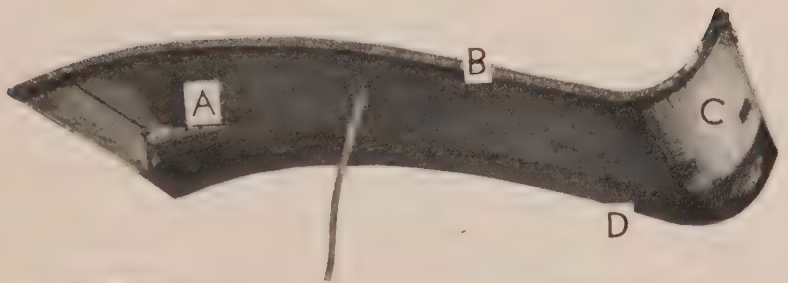
The authors have hitherto studied mid-water trawl fishing and made practical tests on it in sea water. One kind of otter-board used in practice by them is a new type; it is confirmed to be suitable for the small scale mid-water trawl net within 100 m^2 mouth areas. The construction and performance of this otter-board are herein reported.

Photos. 1 and 2 show the whole structure of this otter-board, of which Photo. 1 is a top view, Photo. 2 a rear view. Each board is set up with several thin cedar plates, 27 mm in thickness, to form an S-shaped curvature. The device measures 2 m in length and 1 m in height. The curvature is held by two bent angle iron strips on the upper and lower edges, and steel pipes, 27 mm in diameter, are respectively installed to the front and rear edges to protect from injuries caused by striking floating objects. Two steel plates, having nearly equivalent weight to the buoyancy of the board in sea water, are fitted to the lower part of the board, so the net weight of this board in sea water becomes nearly zero. Thus two good results are obtained of the device not arbitrarily rising up or sinking down in the sea and of the easy hauling of a trawl gear. When the otter-boards are thrown in the sea, they are easily apt to stand upright because of the center of gravity being at the lower part of them. The big radius curvature at the front of the board is made to resemble closely the Clark-Y aerofoil which easily generates a big thrust, on the other hand, the small radius curvature at the rear part serves as a rudder which offers an adequate attack angle to the otter-boards while being towed.

A small tail wing, about 800 cm^2 in area, set in the back center of the rudder, serves as a stabillizer and decreases harmful oscillations of the boards. As the net weight of the board in the air is no more than 40 kg, a single man can easily operate it by himself on the fishing vessel.

This otter-board is towed by only one pennant wire rope shackled to the L-shaped angle-iron, 40 cm in length, which has several holes for the purpose of changing the setting point of the pennant wire and is bolted near the front edge on the center line of the board.

In a preliminary test the writers found the most suitable hole to which to shackle



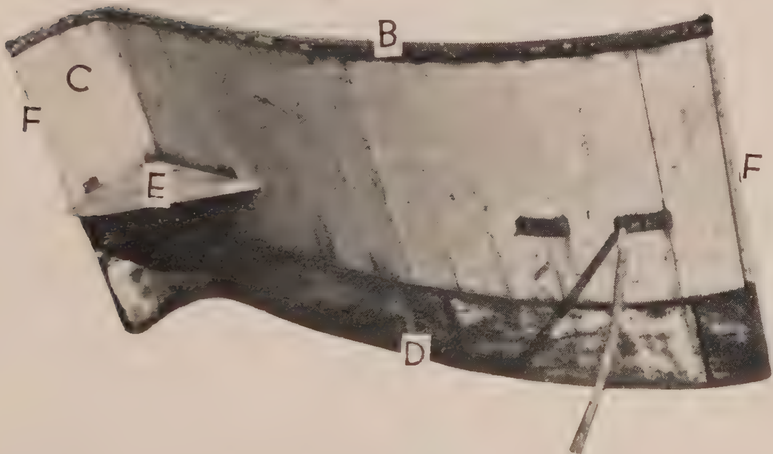


Photo. 1. A top view of this otter-board
Photo. 2. A rear view of this otter-board
A : L-shaped angle-iron to which to shackle the pennant wire
B : Angle-iron strips
C : Rudder
D : Steel plates
E : Tail wing
F : Steel pipes

the pennant wire, which gave the maximum thrust to the otter board. That hole was at about 35 cm distance from the front edge of the otter board. If area and curvature of the rudder are altered, attack angle of the board may be changed, then thrust and resistance may also be changed, so the preliminary test is necessary in order to find the suitable point for the hole to which to shackle the pennant wire. Also in the preliminary test it was found that the thrust force produced by this otter board was about 210 kg and the resistant force was about 140 kg at speed 3 knots.

Acknowledgment

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クジラリパーゼに関する研究

第1報　ミンク膵リパーゼ

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Studies on Whale Lipase

I. Lipase of the pancreas of little finner

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Abstract

Acetone powder of the pancreas of whale (*Balaenoptera acuto-rostrata* LAC.) was prepared in a manner essentially similar to that described by Meyer *et al.* (1947). Whereas for ox-lipase about 20–30 hrs. was required to perform perfect extraction with glycerin from its acetone powder, for whale-lipase only 3 hrs. was needed at 30°C. Optimum pH with Michaelis' ammonium buffer was 8.9 for both lipases. Effects of egg albumin and $CaCl_2$ as activators on lipase action were examined. Lipase of whale pancreas was activated principally with the latter by a 700 % increase. Whale-lipase digested various fish liver oils and whale oils as well as olive oil, but ox-lipase so acted upon fish liver oils less, and whale oils much less than olive oil.

緒　　言

リパーゼに関する研究は Eberle (1834), C. Bernard (1856) により膵液が脂肪分解能を有する事を発見した事に始まり，以後 Willstätter[1–3] 一派を始め多数の報告あり。中，水産動物リパーゼに就てはコウイカ・ヤリイカ・ジャコウダコ[4]，ブダイ[5]，クサフグ・キヌベラ・カエルウオ[6]等の肝膵臓，イソギンチャクの腸間膜[7]，カスベの膵液[8]，サバ・イワシ・ニシン・カツオ・ブリ[9]等の膵臓抽出液，サバ・メンハーデン・アカダイ[10]等の肝臓・幽門垂・腸管等，夥しい報告が見られるが，一部魚類の特有器官である幽門垂ではかなり強力なものから殆んど存在せぬ[11]ものまで魚種により大きく異るようである。

鯨類における臓器酵素としては胃ペプシン[12]，膵トリプシン[13]，肝臓・腎臓カテプシン[14]並びにアルギナーゼ (arginase)[15] 等に就て報告されているが，リパーゼに関する報告は高田[16] の胃リパーゼが第3胃に多く存在（ペプシンは第2胃）[12] する事，膵リパーゼが相当強力な活性を有する事を定性的に観察しているのみで，この他は詳細な報告は見当らぬ。哺乳動物でありながら水棲という環境に適応する為に厚く豊富な脂肪層（体重の約20%前後を占む）に蓄積されている事よりして旺盛な脂質代謝，従ってそのリパーゼも極めて強力なるべき事が予想される。更にまたマッコウクジラのような異常な脂質代謝（多量のワックスと相当量の比較的低級な脂肪酸を含有する）を行っている種属のリパーゼの特異性は興味を惹く所である。これに関し，ワックス[illegible]に対する作用が[illegible]，詳細は細菌学教室坂井教授と共同実験中で別の機会に報告する。

いま1頭当り平均10kgの膵臓として，沿海捕鯨のみで3000〜4000頭，これに北洋・南氷洋捕鯨を加える時は年間優に10000頭を越える捕獲頭数（1958/59年度には実に12000頭に達す）を考えると，インシュリン資源と共に酵素資源としても豊富貴重な存在と考える。ここに牛と対比しつつミンクの膵リパーゼの酵素化学的な性質を観察したので報告する。

実 験 の 部

試　料

北海道網走沖で捕殺したミンク（コイワシクジラ） (*Balaenoptera acuto-rostrata* LAC.) の死後10時間前後の鮮度極めて良好なものを解剖後直ちに凍結輸送，－35°C 附近に凍結貯蔵したものを供試した。この凍結貯蔵物は相当長期にわたつて活性を保持する。牛の膵蔵は屠殺直後の成牛のものを供試。

1. アセトン・パウダー調製

アセトン・パウダーの調製には Willstätter[1] 法と Meyer *et al.*[17] の之の変法があるが主として後者に従つて（溶媒の使用量少量で済む）次のように行つた。凍結貯蔵のミンク膵蔵の脂肪塊及び繊維を出来る限り除去したものを，氷結状態のまま低温下肉挽器に2～3回通してペースト状にする。このもの1kgを取り3倍量の冷アセトンを攪拌しながら少量宛添加（ドライアイスを利用すると便利である），低温に約3時間放置後ブッフナー漏斗で吸引濾過，次に2倍量のアセトンを加えて同様処理を2回繰返した後，冷アセトン・エーテル等量混液2倍量加えて2回，最後に2倍量の冷エーテルで2回処理す。ここに得られた脱水物を更に真空デシケーター内で速やかに乾燥白色粉末とす。このもの低温下真空内貯蔵する時は数年間にわたつて安定である。

収量―ミンク：241 g (24.1%)，牛：215 g (21.5%)

2. 酵素液調製

浸出剤には稀アンモニア液も用いられているが，安定性の遙かに大きいグリセリンを用いて次のように行つた。前記のアセトン・パウダーに16倍量の 87% グリセリンを加え 30°C に一定時間放置後 3500 r. p. m. 40分遠心して半濁液を取り，これに3～5倍量の水を添加後更に30分遠心すると透明なグリセリン浸出液が得られる。

3. 活性測定

Willstätter[1] のアルカリ滴定法により次のように行つた。

オリーブ油（ケン化価 193.0，酸価 1.6，比重 0.9200）2 ml，0.2 N NH_4Cl-NH_4OH 緩衝液 (pH 8.9) 10 ml，2% $CaCl_2$ 液 0.5 ml，3% アルブミン液（武田製卵アルブミン）0.5 ml 及び酵素液 1.0 ml（87% グリセリン抽出原液をそのまま乃至は3～5倍稀釈液を供試）より成る反応混液を 30ml 共栓瓶に取り 40°C 恒温水槽中1時間振盪下消化後，アルコール 30 ml，エーテル 15 ml を以て反応液を洗出し，分解生成した脂肪酸を 0.1 N KOH アルコール溶液によりフェノールフタレインを指示薬として滴定，盲験値を差引き常法により分解度をケン化価に対する百分率として計算す。

一般油脂分解の場合もこれに準ず。

実験結果並びに考察

1. 浸出時間

対照に牛の膵臓を用いてグリセリンのクジラリパーゼ浸出能力を比較した。

牛・ミンク膵臓のアセトン・パウダーには 87% グリセリンを16倍量加え 30°C に於て浸出，オリーブ油の分解度を時間の経過と共に測定した結果が第1表の如くである。活性測定の酵素液はグリセリン抽出原液の3倍稀釈液を供試。

これによるとミンク膵リパーゼは *'Lyo-form'* が主で30°C約3時間で殆んど全部が浸出せられて強力な活性を示すが，牛の膵リパーゼは 30°C 20～30時間を要し *'Desmo-form'* が多量なる事を示す。浸出の難易はこのような酵素の結合形態の他に組織の脆弱性も関係しているものと考えるが，Boissonas[18] (1948) も豚の膵リパーゼの最高浸出には36時間を要する事を報告している。相対的な活性比較の場合にはこのように浸出時間を顧慮するを要す。各組織の最適浸出時間により調製した酵素液を以て測定しなければならない。以下ミンクリパーゼは3時間，牛リパーゼは20時間の浸出液を供試した。

Table 1. Activity and extraction time of acetone powder

Time	Little finner-lipase		Ox-lipase	
	Acidity increase (0.1 N KOH)	Digestion ratio	Acidity increase (0.1 N KOH)	Digestion ratio
hrs.	ml	%	ml	%
0.5	10.15	16.0	1.06	1.7
1	11.38	17.9	3.62	5.7
3	12.12	19.1	4.31	6.8
5	12.24	19.3	6.16	9.7
8	12.06	19.0	6.20	9.8
10	10.20	16.1	6.39	10.1
15	11.13	17.6	6.68	10.8
20	9.90	15.6	7.47	11.8
30	8.80	13.9	7.60	12.0
40	7.95	12.5	6.85	10.8

2. 至適 pH

酵素の至適 pH は標品の純度，緩衝液の種類，基質等により大きな影響を受けるものであるが，殊にリパーゼの場合は乳化性に支配され易い。ここに同一条件下で牛•ミンク両リパーゼの至適 pH を比較して見た。0.2N NH_4Cl–NH_4OH 緩衝液，酵素液 1.0ml（グリセリン抽出原液の5倍稀釈液）について 40°C 1時間消化を行つた結果が第2表に示す通りである。

Table 2. Dependence of digestion of olive oil on pH by whale- or ox-lipase

Lipase	pH		8.04	8.27	8.49	8.67	8.86	9.10
Little finner	Acidity increase (0.1 N KOH)	ml	3.86	3.60	7.02	7.97	9.05	6.45
	Digestion ratio	%	6.1	5.7	11.1	12.6	14.3	10.2
Ox	Acidity increase (0.1 N KOH)	ml	1.39	2.40	3.29	3.98	4.68	2.02
	Digestion ratio	%	2.2	3.8	5.2	6.3	7.4	3.2

これ迄の報告によるとヒマシリパーゼの至適 pH が 4.7〜5.0（発芽に伴い中性附近へ移行す），胃リパーゼ[19,20]は pH 5.5〜8.6（動物の種類，標品の精製度により大きく変移する），乳汁リパーゼ[21]は pH 8.0附近であるのに対し膵リパーゼは pH 8.5〜8.9[1]とされているが本実験条件の下ではミンク・牛共にその膵リパーゼは至適 pH 8.9 を示す。

3. アルブミン及び $CaCl_2$ の影響

これ迄の膵リパーゼに対する代表的な *'activator'* としては，アルカリ性反応に於ける胆汁酸[22)23)]，卵白アルブミン[1 21]，可溶性 Ca 塩[1,25)26)]等が知られているが，これらは基質と酵素の接触を促進する吸着性，乳化作用，及び反応生成物（主として高級脂肪酸）の反応系外への除去などになるものと考えられている。クジラ・牛両リパーゼが卵白アルブミン及び $Ca^{\cdot\cdot}$ によりどの程度影響を受けるかを観察した結果が第3表の如くである。実験Ｉでは 87% グリセリン抽出原液 1.0 ml，実験Ⅱではこの酵素原液の3倍稀釈液 1.0 ml を供試した。

Table 3. The effects of egg albumin and $CaCl_2$ on lipase action

Activator			Little finner-lipase					
			Experiment 1 **			Experiment 2 *		
No.	$CaCl_2$	Albumin	Acidity increase (0.1 N KOH)	Digestion ratio	Degree	Acidity increase (0.1 N KOH)	Digestion ratio	Degree
	mg	mg	ml	%		ml	%	
1	—	—	1.64	2.6	100	2.46	3.9	100
2	10	—	11.39	18.0	692	13.16	20.8	533
3	—	15	2.15	3.4	131	2.73	4.3	113
4	10	15	11.45	18.1	695	13.60	21.5	552

Ox-lipase					
Experiment 1 **			Experiment 2 *		
Acidity increase (0.1 N KOH)	Digestion ratio	Degree	Acidity increase (0.1 N KOH)	Digestion ratio	Degree
ml	%		ml	%	
1.45	2.3	100	2.28	3.6	100
4.68	7.4	322	6.14	9.7	269
1.83	2.9	122	2.53	4.0	111
4.62	7.3	317	6.20	9.8	272

* There was used a original glycerol enzyme solution

** There was used a threefold diluted enzyme solution

両者共に $Ca^{\cdot\cdot}$ による影響非常に大で，クジラリパーゼでは無添加の場合の 550〜700% であるのに対し，牛リパーゼでは 300% 前後に止まる。対照の活性が同程度であるので，クジラリパーゼの方が遙かに $Ca^{\cdot\cdot}$ の影響を大きく受けている事が解る。両者共に卵アルブミンの賦活作用は余り認められない。これに就て Platt & Dawson[27)] もアルブミンの賦活作用を特別に認めていない。但し長時間の消化の場合にはリパーゼの安定性に寄与する為に好影響を与えるようである。

4. 各種油脂類の分解能

構成脂肪酸を定性的・定量的に異にする各種天然油脂に対する各種膵リパーゼの分解能は興味を惹く所であるが，他の加水分解酵素に比較してリパーゼはこの特異性は余り顕著でないとされているが，豚リパーゼ

に就てヤシ油・ヒマシ油・糠油・パーム核油等の植物油分解能大なる事が報告されているので，ミンク並びに牛の膵リパーゼの各種天然油脂に対する分解能を比較してみた。酵素液はグリセリン抽出原液の 5 倍稀釈液を供試。結果は第 4 表の如くである。

Table 4. The hydrolysis of little finner- and ox-pancreas lipase on various natural oils
Digestion ratio for 30, 60 & 180 min. at 40 °C

Oils	Acid Value	Sap. Value	Iodine Value	Little finner-lipase			Ox-lipase		
				30min.	60	180	30min.	60	180
				%			%		
Olive oil	2.1	193.0	80.2	8.4	10.9	15.8	4.3	6.3	8.6
Cotton seed oil	0.8	192.3	107.0	8.6	11.6	16.0	4.2	6.2	8.2
Castor oil	0.99	182.2	85.4	8.0	12.7	18.7	4.9	7.0	10.0
Rice-bran oil	17.8	210.5	102.8	9.2	12.1	17.9	5.5	8.6	12.8
Sardine oil	4.8	192.8	170.5	7.4	10.5	17.6	2.1	3.8	6.2
Pollack liver oil	1.4	185.8	165.0	6.9	10.6	16.3	2.5	4.3	5.3
Cod liver oil	0.63	181.7	165.2	6.0	9.9	15.2	2.0	4.1	5.5
Flat fish liver oil	2.8	179.2	95.0	6.2	10.5	14.9	2.8	3.9	4.9
Shark liver oil	2.1	154.4	113.3	6.5	10.6	13.8	1.9	2.7	3.9
Finback oil	0.4	190.8	111.7	7.1	11.1	16.5	2.3	4.2	5.8
Sperm oil	8.5	127.8	85.8	6.8	10.4	14.0	1.5	1.8	2.7

小野[31]によるとヒマシリパーゼはヒマシ油・オリーブ油等の植物油に対する分解能が，イワシ油・タラ肝油・鯨油等の動物油よりも大で，豚膵リパーゼは逆に動物油に対する分解能の方が大であるとしているが，一方 Hartwell[28]，伊藤[29]，清水[30]等は豚膵リパーゼがヤシ油・ヒマシ油・糠油を強く分解する事を報告している。本実験では，ミンクリパーゼは動物油に対して植物油と大差なく分解するが，牛リパーゼは動物油に対する分解能可なり劣る事が認められる。特に抹香油に対する分解能が小さい。

また Chrzaszes *et al.*[32] によると，豚・牛・羊の膵リパーゼの活性度を大体 4.0：2.5：1.5 としているが，ミンクは牛に対して約 2 倍の活性度を有している。特に抹香油に対する分解能で大差を示し，牛リパーゼはミンクの約½に過ぎない。クジラリパーゼは強力な活性を有する事が認められる。

要　　約

新鮮ミンク膵臓よりアセトン・パウダーを調製し，そのリパーゼの種々の性質を牛膵臓リパーゼと比較した。

1. グリセリンによる浸出に於てミンク膵リパーゼは '*Lyo-form*' が主で 30°C 3 時間で殆んど完了し強力な活性を示すが，牛膵リパーゼは20〜30時間を要し '*Demo-form*' の多い事が認められる。
2. Michaelis の NH_4Cl–NH_4OH 緩衝液を用い，オリーブ油分解に於ける至適 pH を測定し両者共に 8.9 なる事を示した。

3. リパーゼの代表的な *activator* である卵白アルブミン，$CaCl_2$ による賦活作用を検討した所，両リパーゼ共に主として $CaCl_2$ によるもので，その分解度の上昇はミンクに対して約 550〜700 %であるのに対し牛では約 300 %であつた。

4. 各種天然油脂に対する分解能を比較して，ミンクリパーゼは大体オリーブ油と同程度に魚油・鯨油を分解し得るに対し牛リパーゼは明かに劣る。特に牛の抹香油分解能はクジラの約½程度に過ぎない。

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FURTHER STUDIES ON *CIRRULICARPUS GMELINI* (*GRUNOW*) *TOKIDA ET MASAKI*

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Cirrulicarpus Gmelini was first recognized as an independent taxon by Grunow (1867, p. 72) who placed it in the genus ***Kallymenia***. Yendo (1915) transferred the species to *Erythrophyllum*, where it remained until Tokida and Masaki (1956) founded the new genus, ***Cirrulicarpus***, for this taxon.

Tokida and Masaki were the first to record fertile plants of ***Cirrulicarpus***, and they described the tetrasporangiate and cystocarpic specimens. By sectioning the cystocarpic thallus they also observed the carpogonial branch apparatus and a fusion cell. According to Tokida and Masaki there were six cells in the carpogonial branch, linearly arranged in a strong curve so that the basal cell was brought into close proximity with the carpogonium. They suspected that the basal cell served as the auxiliary cell although the carpogonium was not seen fusing with it. A large fusion cell, formed by all the cells of the carpogonial branch was interpreted by Tokida and Masaki to produce gonimoblast cells. Therefore the carpogonial branch system was believed to be a procarp. The sections of ***Cirrulicarpus Gmelini*** illustrated by Tokida and Masaki (figs. 12-17) clearly showed that the carpogonial branch system is located in the inner cortex, and the trichogyne is long and penetrates through the outer cortex. As the cells of the carpogonial branch system enlarge they grow into the medullary region and often occupy a more or less median position inside the thallus.

While the position and organization of the female reproductive apparatus can be observed from sections, it is practically impossible to observe the reproductive structures in their entirety and in isolation from the vegetative cells because the large cells of the female reproductive apparatus are frequently cut and parts of cells or entire cells may be missing from the field. Because the squash technique described by Norris (1957, p. 254) permits entire reproductive structures to be observed, it was used to gain more details about the carpogonial branch apparatus and the development of the gonimoblast in ***Cirrulicarpus Gmelini***. Norris's squash technique employed a solution of ten percent sodium hydroxide as a softening reagent for the thallus, after which the tissue was washed in distilled water and stained with Mayer's acid haemalum. The specimens used for these preparations were collected by Masaki in Kushiro, Hokkaido on December 1, 1956 (dried

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specimen in the herbarium of the University of Minnesota, fig. 1), and on February 15, 1958 (preserved in 70 percent alcohol in the collection of the University of Minnesota). Since the specimens collected in February had few satisfactory stages in the development of the gonimoblast, and the specimen collected in December had many stages, most of the observations reported here were made on the latter specimen.



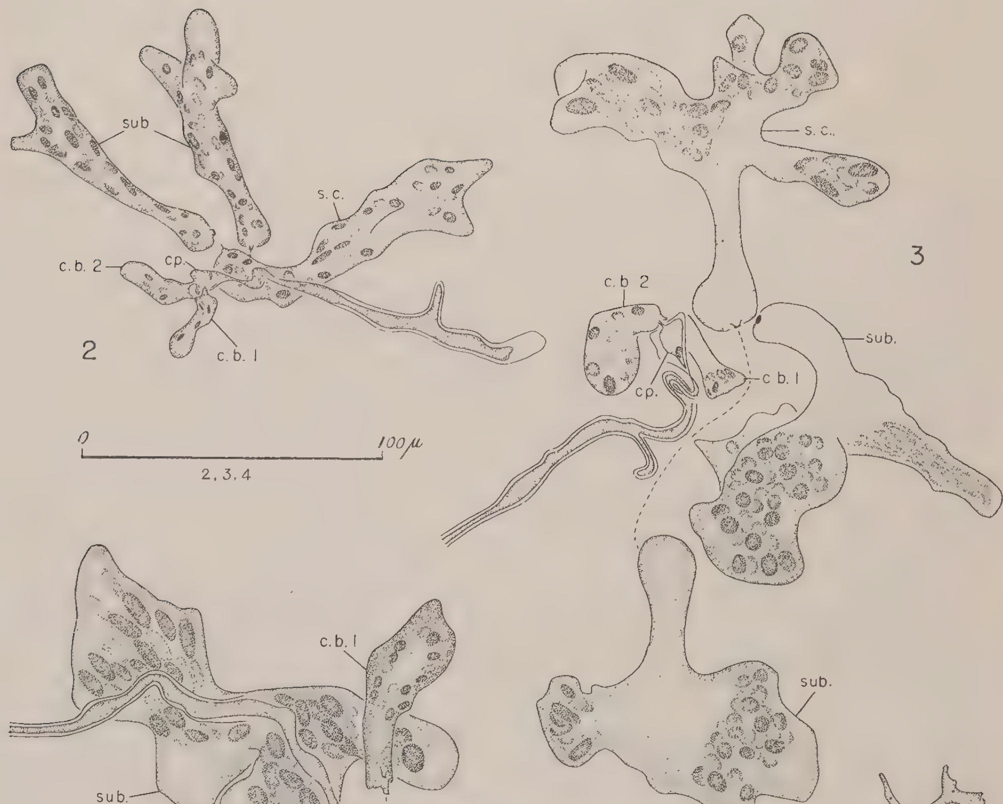
Fig. 1. Female thallus of *Cirrulicarpus Gmelini* collected at Kushiro, 4 Dec. 1956.

From the squash preparations it was found that the carpogonial branch is composed of three cells (fig. 2)*, the terminal one the carpogonium. The trichogyne often has a short branch as in *Erythrophyllum delesserioides*; however, this branch was never fused with any other cell and probably is not involved with the transfer of nuclei to other cells. The first and second cells of the carpogonial branch are similar in size and shape; they are usually oblong-ovate, the narrowed end having the two pit connections very close to one another. Each of these cells usually contains from ten to twenty nuclei. The first cell of the carpogonial branch is connected to a very large cell that often is deeply lobed when mature (fig. 3). This cell is the supporting cell and one of its lobes has several large pit connections where from two to three subsidiary cells in addition to the carpogonial branch are attached. The subsidiary cells are large and often lobed so that they closely resemble the supporting cell; however the supporting cell is slightly larger and more lobed than the subsidiary cells. Each of the subsidiary cells and the supporting cell contain numerous nuclei. As the cells mature they enlarge greatly and the nuclei migrate into the lobes opposite the pit connections. At this stage it is not uncommon to find from 45 to 88 nuclei in each of these large cells.

Stages in the actual fertilization process were not observed, and the nucleus within the carpogonium was seen only a few times. However, in fourteen different female apparatuses the carpogonium was fused with the supporting cell (fig. 4), and in two systems the carpogonium was seen after it had fused with one of the subsidiary cells. It may be hypothesized that the diploid nucleus usually migrates from the carpogonium into the supporting cell, but occasionally it may be transferred from the carpogonium to one of the subsidiary cells.

Subsequent to the transfer of the diploid nucleus into one of the large cells of the carpogonial branch system a fusion cell is formed by the uniting of the subsidiary cells with the supporting cell (fig. 6 & 7). The cells of the carpogonial branch do not join with the fusion cell. Stages in the development of the fusion cell were not evident in the material at hand. The fusion cell enlarges somewhat and at the same time it produces numerous small cells from the ends of the lobes. These small cells elongate rapidly and produce non septate tubes, the connecting filaments, that radiate from the fusion cell (fig. 6). The connecting filaments grow through the medullary tissue for rather long distances. It seemed that only the tips of the connecting filaments contained an active protoplast, the remaining part of the tube usually appeared to be nearly devoid of protoplasm. Oftentimes vegetative cells were laterally attached to the connecting filaments which may indicate that they provide supplementary nourishment for the growth of the long tubes.

*The illustrations were prepared by Miss Wilma Monserud of the Department of Botany, University of Minnesota.


sub
s. c.
c. b. 2
cp.
c. b. 1
2
0
100μ
2, 3, 4
s. c.
3
c. b 2
sub.
c b. 1
cp.
sub.
c. b. 1
sub.
c. b 2
cp.
4
sub.
s. c.
5
0
100μ

Diploid nuclei are probably present within the terminal parts of the connecting filaments. When the apices of these filaments come into contact with young, unfertilized carpogonial branch systems they fuse with the supporting cell or one of the subsidiary cells and one or the other of these cells serves as the auxiliary cell. The fusion seems to be a stimulus to the growth of the connecting filament which then enlarges and produces many lobes (fig. 8 & 9). At the same time the connecting filament and the auxiliary cell cut off several large cells that become filled with dense protoplasm (fig. 9). These cells are gonimoblast initials and they divide to produce gonimoblast filaments that radiate throughout the immediate area. Eventually these gonimoblast filaments develop clusters of carposporangia near their apices. The connecting filament may continue to grow through the medulla after diploidization of the auxiliary cell.

According to Tokida and Masaki the cystocarps of *Cirrulicarpus Gmelini* are small elliptical rings that are elongated in the direction of the frond axis. The center of the ring probably was the location of the fusion cell. Since the vegetative cells soon grow into the fusion cell, apparently digesting it, there is little left of the fusion cell by the time the carposporangia have matured. The clear interior region of the ring represents at least part of the length that the connecting filaments grew before they fused with auxiliary cells. As the gonimoblasts from each of the diploidized auxiliary cells developed they fused with one another to form an elliptical ring. Therefore the cystocarps of *Cirrulicarpus Gmelini* are probably compound structures resulting from the development of adjacent gonimoblasts that fused together. Since fertilization and diploidization probably occur only in the young growing parts of the frond, the elliptical shape of the cystocarps seems to be caused by growth of the thallus after diploidization. As was noted by Tokida and Masaki, it is common to observe adjacent cystocarps confluent with one another. In *Cirrulicarpus Gmelini* it is impossible to interpret the cystocarp as the product of one diploidization or even a single fertilization.

The morphology and arrangement of the cells in the monocarpogonial branch apparatus of *Cirrulicarpus Gmelini* emphasizes Tokida's and Masaki's conclusion that it belongs in the Kallymeniaceae. The general morphology of the thallus, the anatomy of the thallus and the monocarpogonial female apparatus with a carpogonial branch of only three cells are characteristics that ally the genus with *Erythrophyllum*. In the Kallymeniaceae, *Erythrophyllum delesserioides* and *Cirrulicarpus Gmelini* are the only

Fig. 2. Carpogonial branch system before fertilization. Fig. 3. Carpogonial branch system, after fertilization. Fig. 4. Carpogonial branch system, after fertilization; the carpogonium has made a connection with the supporting cell. Fig. 5. Two large vegetative cells of the medulla that become filled with a dense proteinaceous material. c.b.1, first cell of carpogonial branch; c.b.2, second cell of carpogonial branch; cp., carpogonium; s.c., supporting cell; sub., subsidiary cells.

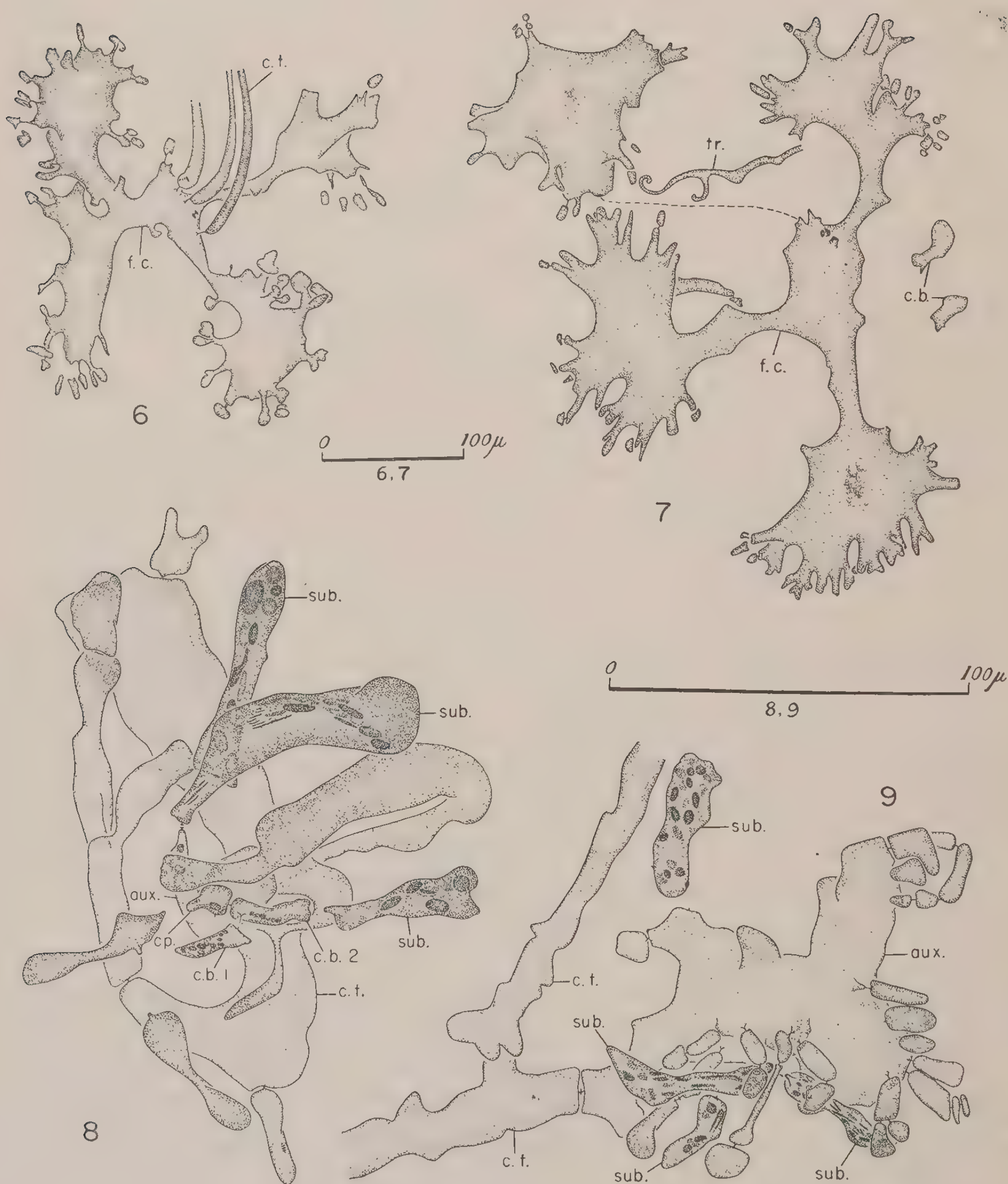


Fig. 6. Fusion cell with three connecting filaments attached to it. Fig. 7. Fusion cell with remnants of the carpogonial branch. Fig. 8. Auxiliary cell system shortly after fusion with the connecting filaments. Fig. 9. Auxiliary cell system, the auxiliary cell has enlarged and is producing gonimoblast initial cells. aux., auxiliary cell; c.b., cells of the carpogonial branch; c.f., connecting filaments; cp., carpogonium; f.c., fusion cell; sub., subsidiary cell; tr., trichogyne.

species that have been investigated in which the first cell of the carpogonial branch does not become part of the fusion cell. Since *Cirrulicarpus Gmelini* does not produce its reproductive organs in specialized papillae as in *Erythrophyllum delesserioides*, there seems to be good justification for maintaining the taxa in separate genera.

Cirrulicarpus exhibits two characteristics that suggest it is probably primitive in relation to other genera in the Kallymeniaceae: 1) a filamentous medulla with little enlargement of sub-cortical cells; 2) a carpogonial branch on the auxiliary cell system. In the latter characteristic it differs from *Erythrophyllum delesserioides* but agrees with *Pugetia fragilissima* (see Norris 1957, fig. 22).

The large cells in the medulla that are filled with a yellowish proteinaceous substance as noted by Tokida and Masaki, may also indicate the primitive position of *Cirrulicarpus* in the Kallymeniaceae, since similar cells are also present in other primitive genera: *Kallymenia*, *Glaphyrymenia* and *Erythrophyllum*. Ruprecht (1851, p. 267) found cells similar to these in *Crossocarpus lamuticus* RUPR. It is possible that the cells illustrated by Ruprecht (Pl. 14, fig. ac) were parts of carpogonial branch apparatuses rather than vegetative cells. However, Yendo (1915, p. 234) reported "long and fusiform" cells in the medulla of *Crossocarpus lamuticus* that may be similar to those found in *Cirrulicarpus*. In addition to these cells Yendo reported that many smaller "clavate" cells were present. It is possible that the smaller cells may have been parts of female reproductive apparatuses.

Norris interpreted vegetative stellate cells in *Kallymenia reniformis* to have a mechanical function in lending strength to the thallus and resistance to tearing. Although the large medullary cells in *Cirrulicarpus* are seldom stellate, they often branch and are usually attached to one another. A mechanical function as well as a possible function in food reserve or translocation of nutritive substances may be attributed to them. Two of these large cells as they appeared in the squashed preparations are illustrated in fig. 5.

Summary

Additional information is provided on the structure of the carpogonial branch apparatus and development of the gonimoblast for *Cirrulicarpus Gmelini* (GRUNOW) TOKIDA ET MASAKI. The auxiliary cell system is isolated from the carpogonial branch apparatus, but they are identical in structure and each is monocarpogonial. The carpogonial branch consists of three cells. Development of compound cystocarps in *Cirrulicarpus* is considered. The information provided supports Tokida and Masaki in their conclusion that *Cirrulicarpus* belongs in the Kallymeniaceae.

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STUDIES ON THE MELOBESIOIDEAE OF JAPAN. III

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4. Dermatolithon dispar (FOSLIE) FOSLIE

Pl. I, Fig. 1, Pl. II, Figs. 1-3, Pls. III & IV

Foslie, 1909, p. 58; De Toni, 1924, p. 667; Mason, 1953, p. 343; Dawson, 1955, p. 276; Scagel, 1957, p. 152; Dawson, Neushul & Wildman, 1960, p. 56, pl. 21, fig. 1.

Syn. *Lithophyllum tumidulum FOSLIE* f. *dispar FOSLIE*, 1907, p. 29; Nichols, 1909, p. 357. *Lithophyllum (Dermatolithon) dispar FOSLIE*, 1909, p. 50. *Lithophyllum dispar (FOSLIE) FOSLIE*, 1919, p. 33, pl. 72, fig. 14. *Fosliella dispar (FOSLIE) G. M. SMITH*, 1944, p. 225; Doty, 1947, p. 170.

Japanese name. Norimaki-modoki (n. n.).

Habit and distribution, in literature. – Epiphytic on *Gelidium arborescens*, *G. nudifrons* (?)*, *Bossiella* sp., *Corallina* sp., *Prionitis Lyallii*, *Ahnfeltia gigartinoides*, *A. plicata*, *Gymnogongrus linearis*, and *Gigartina volans*. Pacific coast of North America, from Northern Washington to California.

Specimens collected. – Nanaehama, near Hakodate, on *Grateloupia filicina* and *Rhodoglossum pulchrum*, June and October 1959, Masaki.

Thallus epiphytic on various red algae, firmly adherent to and partly encircling the host, at first orbicular, later becoming confluent with each other and irregular in shape, reddish purple in color, ca. 1 cm in maximum diameter, to 200-500 μ thick, surface smooth, polystromatic but marginal portion here and there narrowly monostromatic, cells of marginal portion arranged radially in surface view, 12-84 μ long by 11 μ diam.; hypothallium consisting of cells obliquely elongated, 15-84 μ long by 8-11 μ diam., sometimes showing abrupt change in length of cells; perithallium polystromatic, consisting of 7-9 layers of cells in the neighborhood of conceptacles, cells 13-70 μ long by 7-11 μ diam.; cells of epithallium triangular in the marginal portion of thallus but rectangular in the inner, 3-5 μ high, 5-10 μ diam.; secondary pits present but coalescence of cells absent; sporangial conceptacles immersed, sometimes slightly convex, 210-273 μ diam., (110-)126-170(-184) μ high, tetrasporangia 64-112 μ long, 25-63 μ diam., 6-9 sporangia standing on the periphery of floor in each conceptacle, central part of conceptacle floor slightly upheaved and surmounted by a number of elongated cells which connect the floor and the gelatinous plug that closes the conceptacle pore; procarpic conceptacles immersed, up to ca. 105 μ diam. by 63 μ high, cystocarpic conceptacles sometimes slightly convex, 210-252 μ diam., 126-168 μ high, orifice with well developed papillae; spermatangial conceptacles immersed deeply or immediately below the thallus surface, sometimes slightly

*Cf. Dawson *et al.*, 1960, pl. 21, fig. 1.

convex, 85–115 μ diam., 46–84 (–105) high, orifice sometimes provided with a spout, spermatangia 5–7 μ long by 2 μ diam., not at the end of sterigmata.

The writers' sporangial and male specimens agree well in general with the descriptions of the present species given by the authors cited above, except in having somewhat smaller sporangial conceptacles and sporangia and somewhat higher spermatangial conceptacles. Female plants of this species have been previously unknown, but the writers have fortunately discovered procarpic and cystocarpic specimens among their materials as described above.

5. **Dermatolithon canescens** (FOSLIE) FOSLIE

Pl. I, Figs. 2 & 3, Pl. II, Figs. 4–7, Pl. V

Foslie 1909, p. 58; De Toni, 1924, p. 666.

Syn. Melobesia (Heteroderma) canescens FOSLIE, 1900, p. 6; Yendo, 1902, p. 186; De Toni, 1905, p. 1769. *Lithophyllum (Dermatolithon) canescens FOSLIE*, 1905, p. 8; 1909, p. 48. *Lithophyllum canescens FOSLIE*, 1919, p. 32, pl. 72, fig. 3; Yendo, 1916, *in* Okamura, 1916, p. 128; 1936, *in* Okamura, 1936, p. 511.

Japanese name. Sôhan (Yendo).

Habit and distribution, in literature. – Epiphytic on *Padina arborescens*. Pacific coast of middle Honshû, Japan.

Specimens examined. – Epiphytic on the hapteres of *Alaria crassifolia*: Shirikishinai, near Hakodate, November 1958, Yakushi. On *Padina arborescens*: Enoshima, Kanagawa Pref., April 1898, Okamura (Alg. Jap. Exsic. No. 39); Misaki, Kanagawa Pref., April 1900, Yendo (Herb. Fac. Agr., Hokkaido Univ.); Kominato, Chiba Pref., 28 August 1959, Iwamoto.

Thallus epiphytic on brown algae, firmly adherent to the host, forming suborbicular patches, which later become confluent with each other, 2–10 cm in diam., color of the crust purplish red when fresh, whitish rose on drying, monostromatic at margins, polystromatic in the inner portion, usually up to 120 μ thick, sometimes up to 200–300 μ; hyothallium monostromatic, cells obliquely elongated, 12–55 μ long, (7–) 9–16 μ diam., showing marked differences in length in one and the same individual; perithallium consisting of 2–5 layers of cells of various length, cells 10–42 μ long, 7–13 μ diam.; secondary pits present between each vertical cell row, but coalescence of cells absent; cells of epithallium triangular, 5 9 μ diam. by 3–5 μ high; sporangial conceptacles immersed, 252–268 μ in inside diameter, 63–105 μ high, tetrasporangia 42–84 μ long, 25–42 μ diam., 5–7 sporangia standing on the periphery of floor in each conceptacle, central part of conceptacle floor slightly upheaved and surmounted by a number of hairy cells surrounded by mucilaginous substance, orifice without papillae; female conceptacles immersed, nearly flat on surface, mature but unfertilized conceptacles 105–126 μ diam., 63–84 μ high, cystocarpic conceptacles

nearly flat on surface, 55 210 μ diam., 84 126 μ high, orifice with well developed papillae; spermatangial conceptacles slightly elevated on surface, (55)84-126 μ diam., 42-63 μ high, opening by a narrow pore sometimes extended into a spout, sometimes embedded deeply in the tissue without an opening, spermatangia narrowly cylindrical in shape, ca. 3 μ diam. by 9 μ long.

In the present species, tetrasporangial conceptacles have been described by Foslie (1900, 1909) and Yendo (1916, 1936), but cystocarpic and spermatangial ones have not been reported by anyone. The writers fortunately could observe not only sporangial conceptacles but also female and male ones in their specimens epiphytic on *Alaria* collected near Hakodate. These specimens were confirmed to agree well with the above listed specimens of Yendo and Iwamoto. Yendo's specimen was collected at Misaki, the type locality of the present species, and was identified as *Melobesia canescens FOSL.* by Yendo himself. The writers' hearty thanks are due to Professer D. Murayama of the Faculty of Agriculture, Hokkaido University, for the loan of Yendo's specimen, and to Mr. K. Iwamoto of Tokyo Fisheries University for his kindness in collecting and sending the specimens from Kominato in compliance with the writers' request.

6. **Melobesia farinosa** LAMOUROUX

Pl. I, Figs. 4 & 5, Pl. II, Figs. 8-12, Pls. VI & VII

Lamouroux, 1816, p. 515; Kützing, 1849, p. 696; Rosanoff, 1866, p. 69; Solms-Laubach, 1881, p. 11; Ardissone, 1883, p. 445; Hauck, 1885, p. 263; Foslie, 1900, p. 20; 1905, p. 96; Yendo, 1902, p. 186, 1916, *in* Okamura, 1916, p. 125, 1936, *in* Okamura, 1936, p. 506; De Toni, 1905, p. 1764, 1924, p. 645; Balakrishnan, 1947, p. 305, 36 text-figs., pl. 1; Rayss, 1959, p. 20.

Syn. Fosliella farinosa (LAMOUROUX) HOWE, 1920, p. 587; Taylor, 1937, p. 270, 1939, p. 10, 1957, p. 252.

Japanese name. Ibc-mokasa (n.n.).

Habit and distribution, in literature. – Epiphytic on *Caulerpa Freycinetii*, *Caulerpa* sp., *Valonia ventricosa*, *Padina paronia*, *Spathoglossum* sp., *Ecklonia radiata*, *Fucus linifolius*, *F. serratus*, *Cystoseira a ticulata*, *C. granulata*, *C. myrica*, *Hormophysa* sp., *Sargassum crispum*, *S. herbaceum*, *S. Horschuchii*, *S. linifolium*, *S. Turneri*, *Sargassum* sp., *Turbinaria* sp., *Chondrus crispus*, *Phyllophora nervosa*, *Rhodymenia palmata*, *R. phyllophora*, *Bryothamnion triangulare*, *Laurencia obtusa*, *L. papillosa*, *Laurencia* sp., *Cymodocea manatorum*, *C. serrulata*, *Potamogeton* sp., *Zostera marina*, *Zostera* sp. Middle Honshû, Japan (?)*, Ryûkyû (?)*, Formosa (?)*, China Sea; Celebes Sea; Malay Archipelago; Indian Ocean; Pacific coast of N. America; South Pacific Ocean; Australia; Caribbean Sea; West Indies; Atlantic coasts of N.

*Cf. Yendo, 1916. p. 125, 1936, p.507.

America, Europe and Africa; Canaries; Mediterranean Sea; Adriatic Sea; Red Sea.

Specimens collected. Epiphytic on the leaves of *Sargassum confusum*: Nanaehama, near Hakodate, December 1958, Masaki; Oshoro, 24 November 1959, Yamazaki. On *Ecklonia Kurome:* Nou, Niigata Pref., 29 November 1959, Saito. On *Padina arborescens*: Misaki, Kanagawa Pref., 8 April 1960, Tokida. On *Dictyopteris undulata* and *Sargassum* sp., Shirahama, Wakayama Pref., 30 March 1960, Tokida.

Thallus epiphytic on various larger brown algae, firmly adherent to the host, at first suborbicular and 2–4 mm diam., later becoming confluent and overlapping, purplish red in color when fresh, monostromatic in vegetative part, cells in marginal portion 12–20 μ long by 5–9 μ diam. in surface view, 5–13 μ high by 7–13 μ diam. in section, cell-fusion frequent, each cell except those along the thallus margins and trichocyst initials obliquely cut off a cover cell, cover cells rectangular to roundish in surface view and triangular in section, trichocyst initials elongate, 22–25 μ long by 7–10 μ diam.; tetrasporangial conceptacles hemispherical, 63–126 μ high, (84–)126–168(–189) μ diam., tetrasporangia 40–80 μ long, (20–)25–50(–63) μ diam., 3–7 sporangia standing on the periphery of floor of each conceptacle, always tetrasporic in the writers' material, central part of conceptacle floor occupied by a columella, roof of conceptacle thin, composed of one to two layers of cells; female conceptacles hemispherical, 45–55 μ high, 45–60 μ diam. when young, (25–)42–63(–91) μ high, (29–)105–126 μ diam. when carpospores are formed, provided with papillae on the inner wall of pore, carpospores arising from periphery of floor; spermatangial conceptacles convex, (29–)33–46(–55) μ high, (42–) 55–84 μ diam., spermatangia narrowly cylindrical in shape, 3–7 μ long by 2 μ diam., while attached to their mother cells, but ellipsoidal to globular with no appendage when detached and set free in the conceptacle cavity.

The above description is based chiefly on the specimens growing on the leaves of *Sargassum confusum* from Nanaehama. It agrees well in general with tbe descriptions of the present species given by the authors cited above, except in somewhat smaller dimensions of both tetrasporangial and cystocarpic conceptacles. (Cf. Foslie, 1905, p. 98; Taylor, 1957, p. 252).

Melobesia farinosa LAMX. has already been listed in the marine flora of Japan (Yendo, 1902, 1916, and 1936). However, Yendo commented under the present species in his treatment on the Corallinaceae published in 1916 to the following effect. "On examining the specimens preserved in the Berlin Botanical Museum, it was revealed that: Marten's specimens from Yokohama and Warburg's from Ryûkyû and Formosa, both reported to be identical with this species, were epiphytic on the leaves of *Zostera marina* and they seemed to be referable to '*Heteroderma zostericola FOSL.*'; Marten's specimens reported from Yokohama under the name of *Melobesia granulata MENEGH*. were attaching to the stipe of *Gracilaria Textorii* and they seemed to be identical with '*Melobesia canescens*

FOSL.' or other related species. As far as I have investigated to date, it is doubtful whether *Melobesia farinosa LAMX.* is distributed in Japan or not."

In view of the above opinion, the occurrence of *Melobesia farinosa* in Japan is believed to be firmly established for the first time in the present report.

Summary

1. *Dermatolithon dispar* (*FOSL.*) *FOSL.* which has previously been known from the Pacific coast of North America is reported herein to be new to Japan on the basis of specimens collected in Hokkaido. The species in Hokkaido is represented not only by the previously known sporangial and male plants but also by the female plant.

2. *Dermatolithon canescens* (*FOSL.*) *FOSL.*, a species previously known only from the Pacific coast of middle Honshû, Japan, and reported to be epiphytic on *Padina arborescens*, is described herein to be found also in Hokkaido growing on the hapteres of *Alaria crassifolia.* Besides the previously known tetrasporic plant, both the male and female plants were also observed and described.

3. *Melobesia farinosa LAMX.*, a cosmopolitan species, which has long been listed in the marine flora of Japan but not without a doubt (cf. Yendo, 1916), is reported herein to have been collected on both coasts of middle Honshû, and also in Hokkaido.

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EXPLANATION OF PLATES

PLATE I

Dermatolithon dispar (FOSLIE) FOSLIE

Fig. 1. Habit of plant growing on *Grateloupia filicina* from Nanaehama, Hakodate ×3

Dermatolithon canescens (FOSLIE) FOSLIE

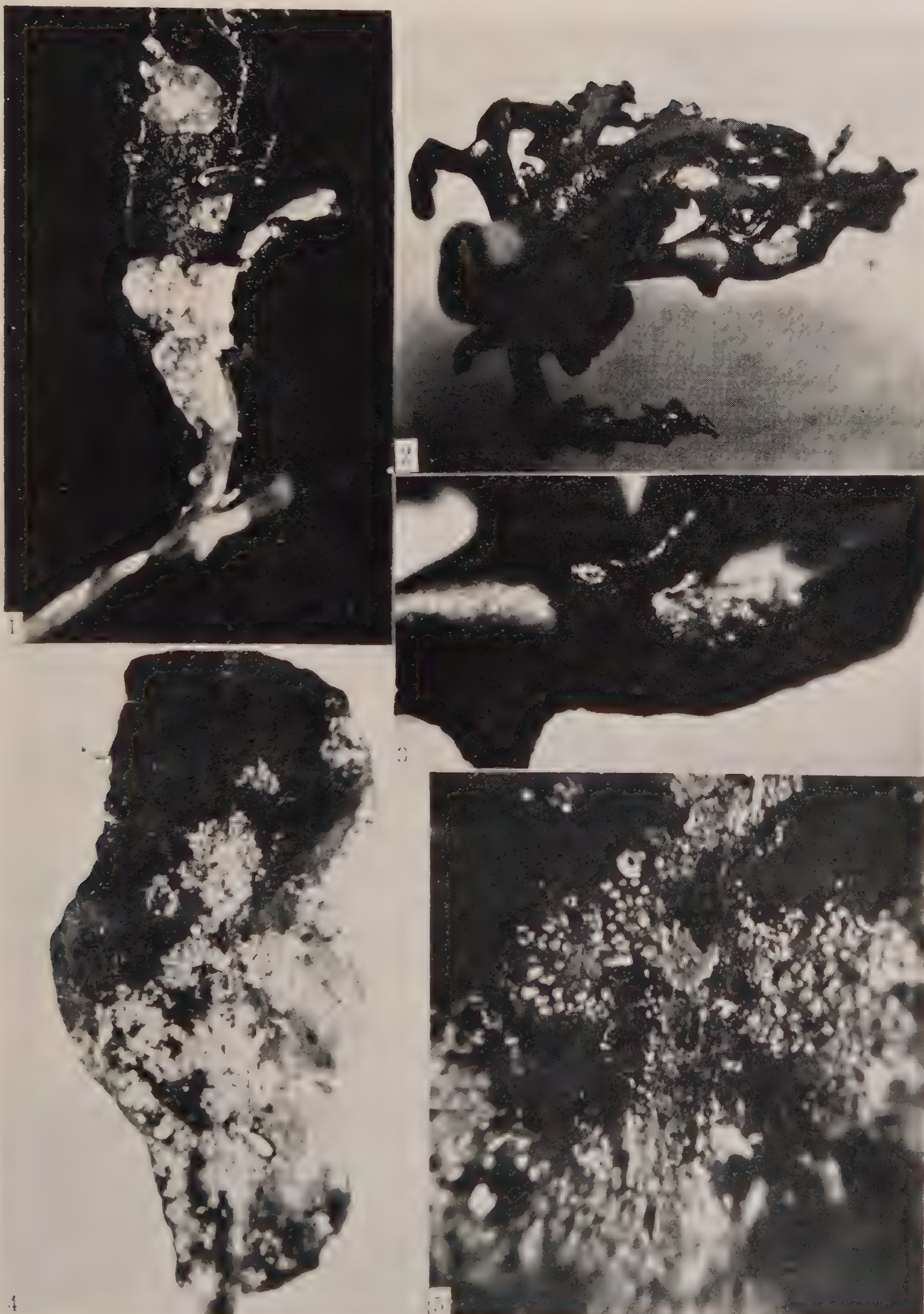
Fig. 2. Habit of plant attached on the hapteres of *Alaria crassifolia* from Shirikishinai ×1

Fig. 3. Enlargement of a portion of the specimen shown in Fig. 2

Melobesia farinosa LAMOUROUX

Fig. 4. Habit of plant growing on a leaf of *Sargassum confusum* from Nanaehama, Hakodate ×2

Fig. 5. Enlargement of a portion of the specimen shown in Fig. 4 ×6



T. Masaki & J. Tokida: Studies on the Melobesioideae of Japan. III

PLATE II

Dermatolithon dispar (Foslie) Foslie

Photomicrographs of vertical sections of plant × 62

Fig. 1. Tetrasporangial crust

Fig. 2. Procarpic and cystocarpic crust

Fig. 3. Spermatangial crust

Dermatolithon canescens (Foslie) Foslie

Photomicrographs of vertical sections of plant × 62

Fig. 4. Tetrasporangial crust

Fig. 5. Procarpic crust

Fig. 6. Cystocarpic crust

Fig. 7. Spermatangial crust

Melobesia farinosa Lamouroux

Photomicrographs of surface viws (Figs. 8 & 9) and vertical sections (Figs. 10–12) of plant

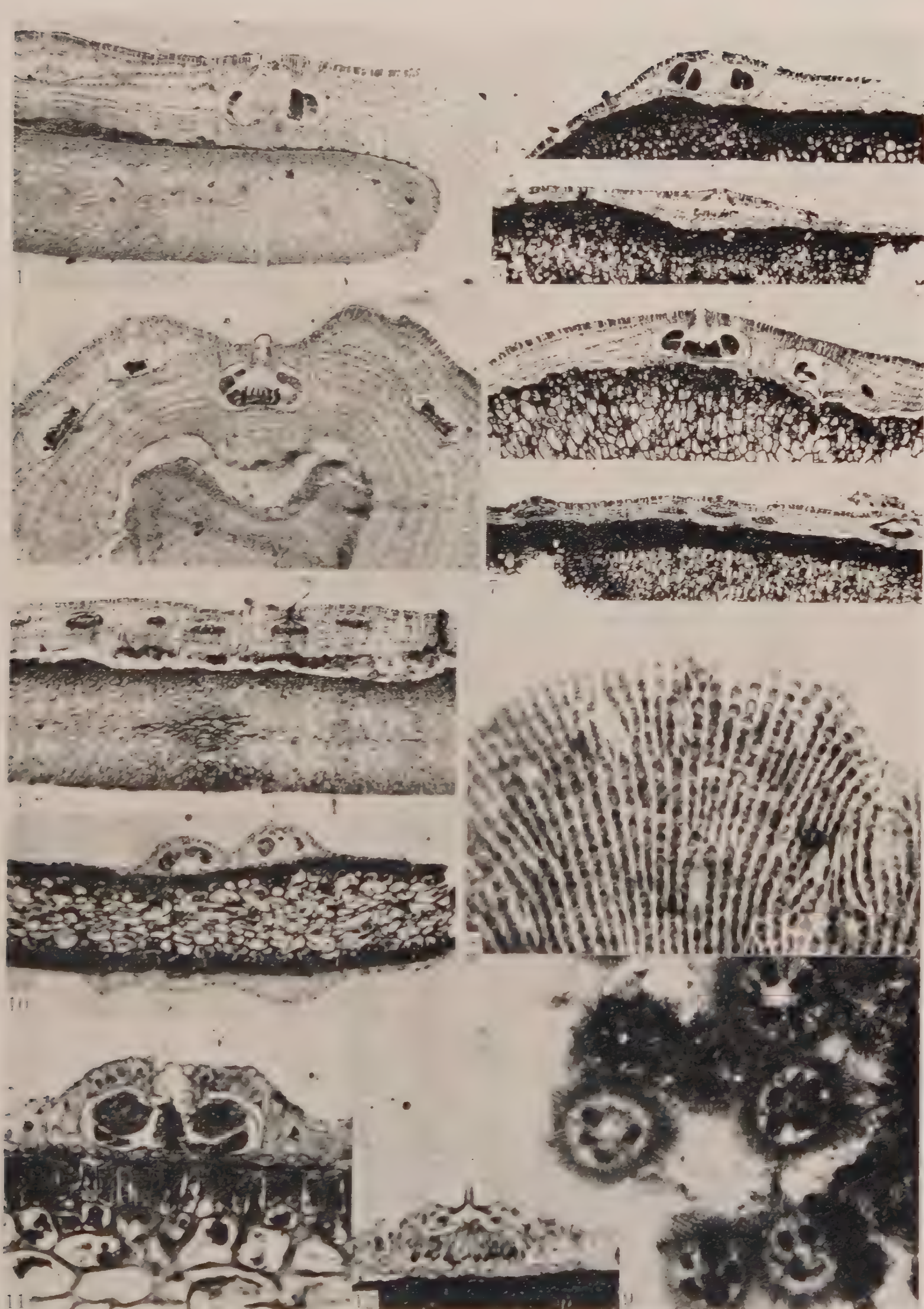
Fig.8. Marginal portion of a crust showing arrangement of the cells and scattered pale trichocysts ×227.5

Fig. 9. Sporangial crust ×62

Fig. 10. Sporangial conceptacles ×62

Fig. 11. Cystocarpic conceptacle ×227.5

Fig. 12. Spermatangial conceptacle ×227.5



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PLATE III

Dermatolithon dispar (FOSLIE) FOSLIE

Fig. 1 & 2. Vertical section through marginal portion of a crust ×400

Fig. 3. Tetrasporangial conceptacle at a younger stage ×275

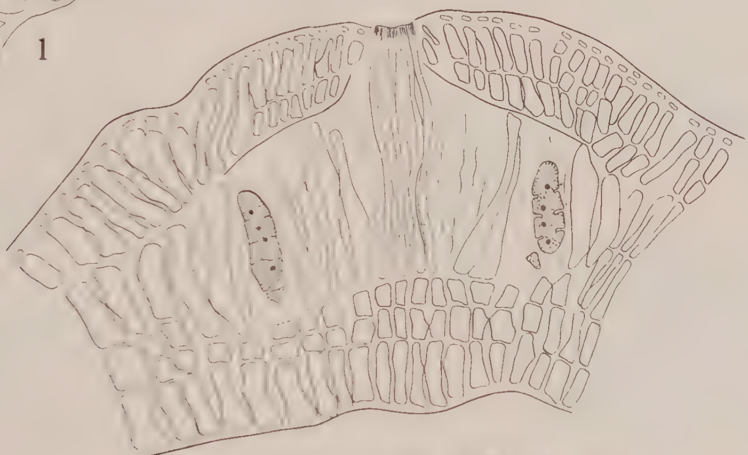
Fig. 4. Tetrasporangial conceptacle at a fully developed stage ×275



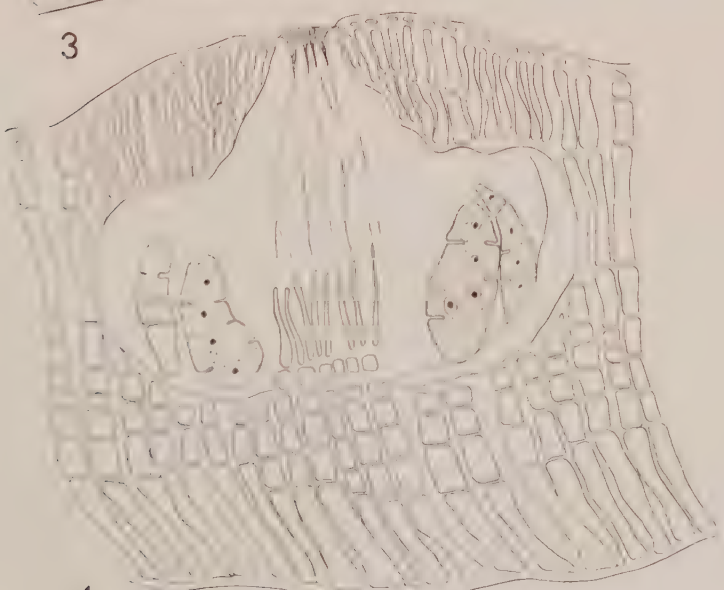
1



2



3



4

PLATE IV

Dermatolithon dispar (FOSLIE) FOSLIE

Vertical sections of conceptacles

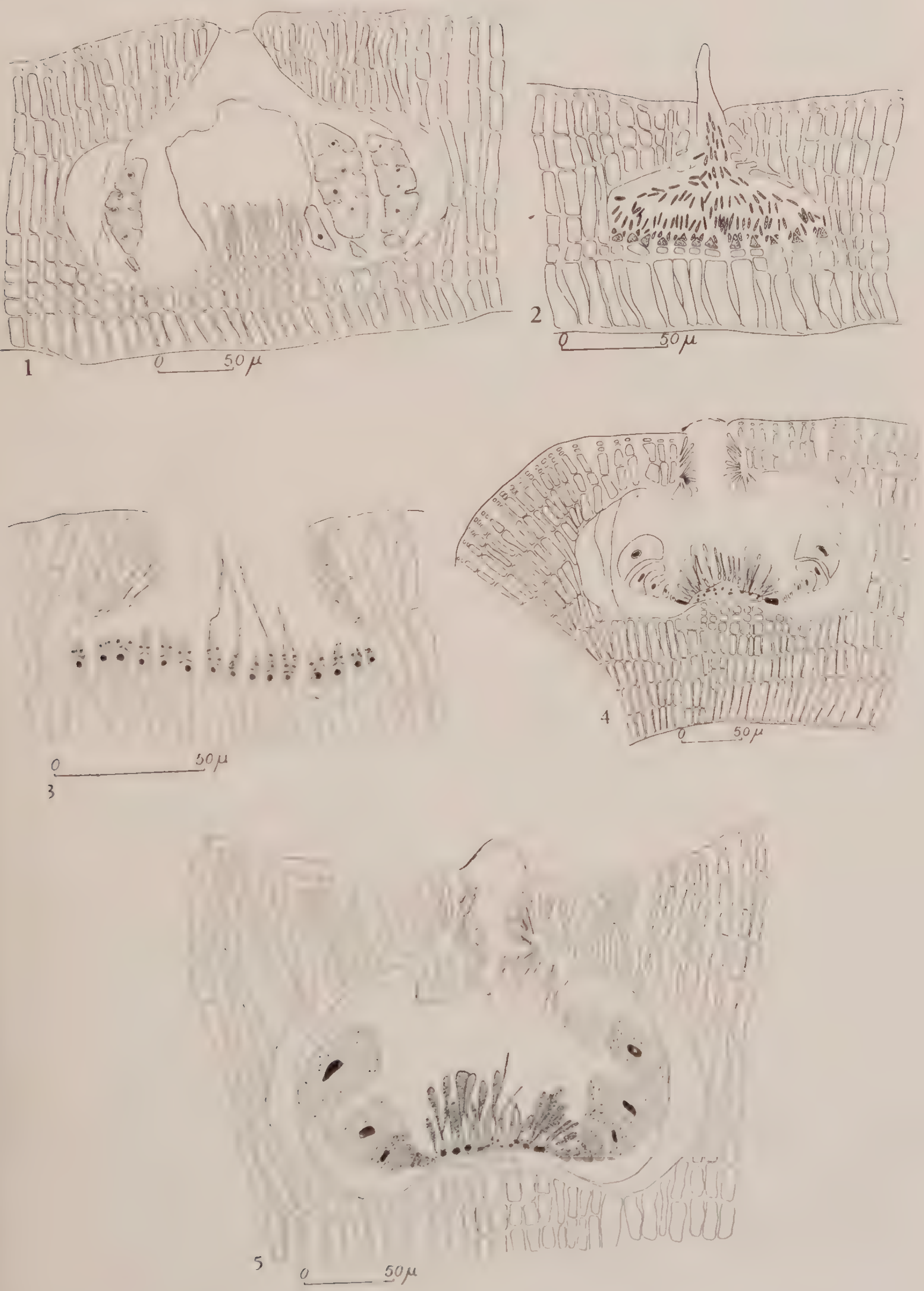
Fig. 1. Tetrasporangial conceptacle

Fig. 2. Spermatangial conceptacle

Fig. 3. Procarpic conceptacle

Fig. 4. Cystocarpic conceptacle, showing well developed papillae

Fig. 5. Cystocarpic conceptacle, showing a lid-like cover on the pore



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PLATE V

Dermatolithon canescens (FOSLIE) FOSLIE

Fig. 1 & 2. Vertical section of the marginal mono- and distromatic portions of a crust ×650

Fig. 3. Tetrasporangial conceptacle

Fig. 4. Spermatangial conceptacle

Fig. 5. Procarpic conceptacle

Fig. 6. Cystocarpic conceptacle

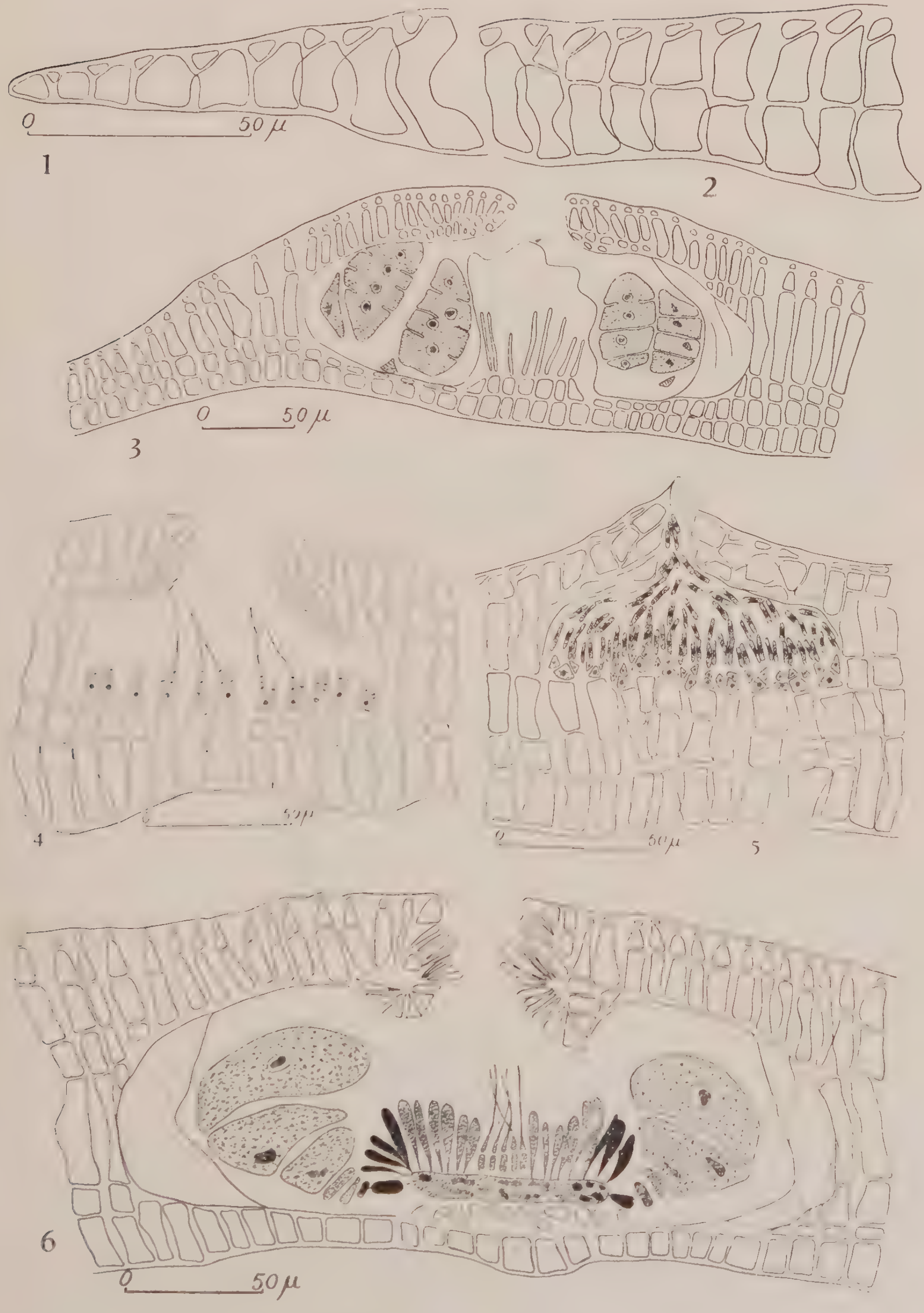

0
50 μ
1
2
0
50 μ
3
50μ
4
0
50μ
5
6
0
50 μ

PLATE VI

Melobesia farinosa LAMOUROUX

Fig. 1. Vertical section of a crust showing a trichocyst ×650

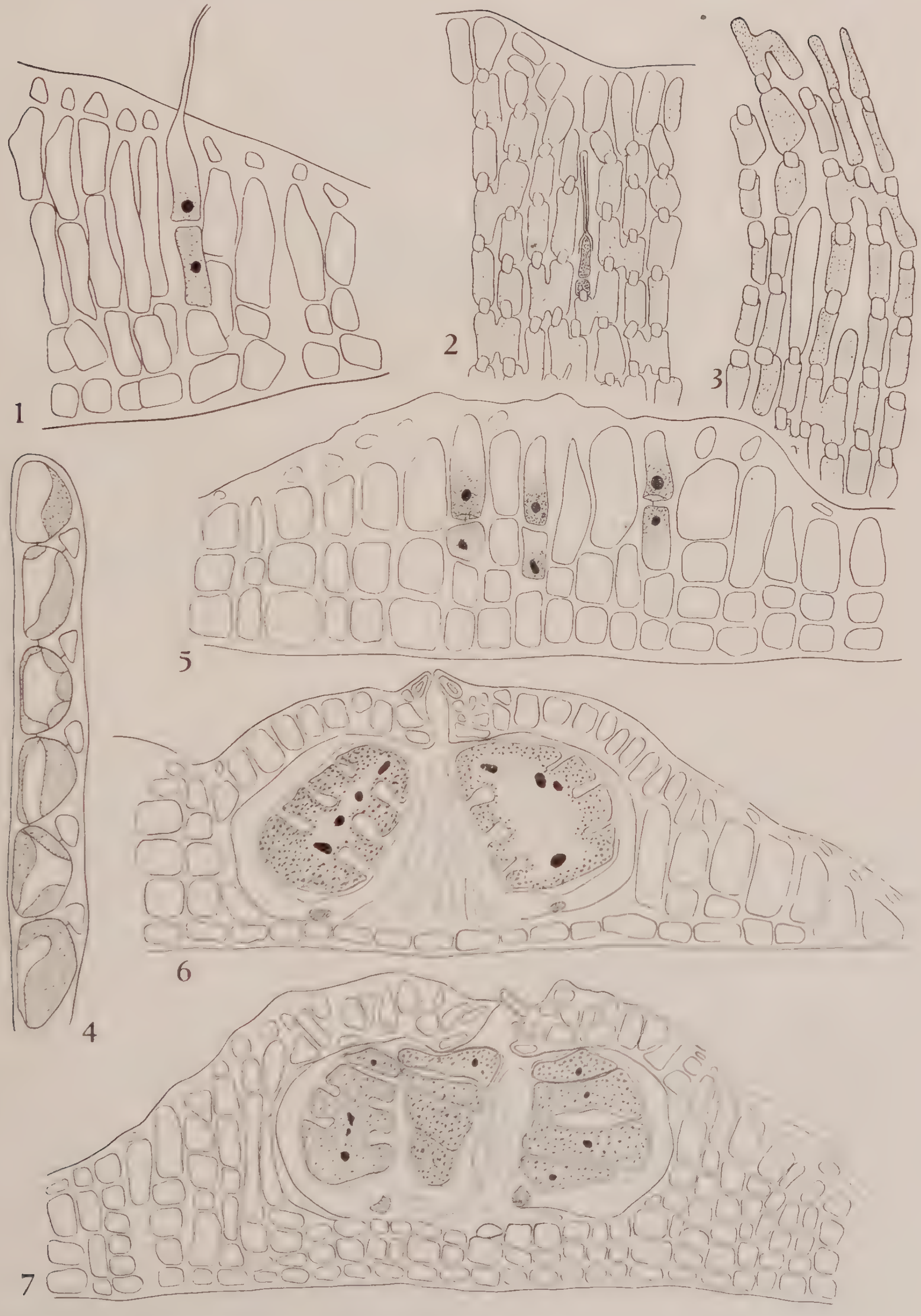
Fig. 2 & 3. Surface view of marginal portion of a crust showing a trichocyst (Fig. 2) and a pale elongated cell or trichocyst initial (Fig. 3) ×650

Fig. 4. Vertical section through marginal monostromatic portion of a crust ×1000

Fig. 5. Vertical section through a crust showing tetrasporangium initials ×650

Fig. 6. Tetrasporangial conceptacle with a monostromatic floor ×400

Fig. 7. Tetrasporangial conceptacle with a polystromatic floor ×400



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PLATE VII

Melobesia farinosa LAMOUROUX

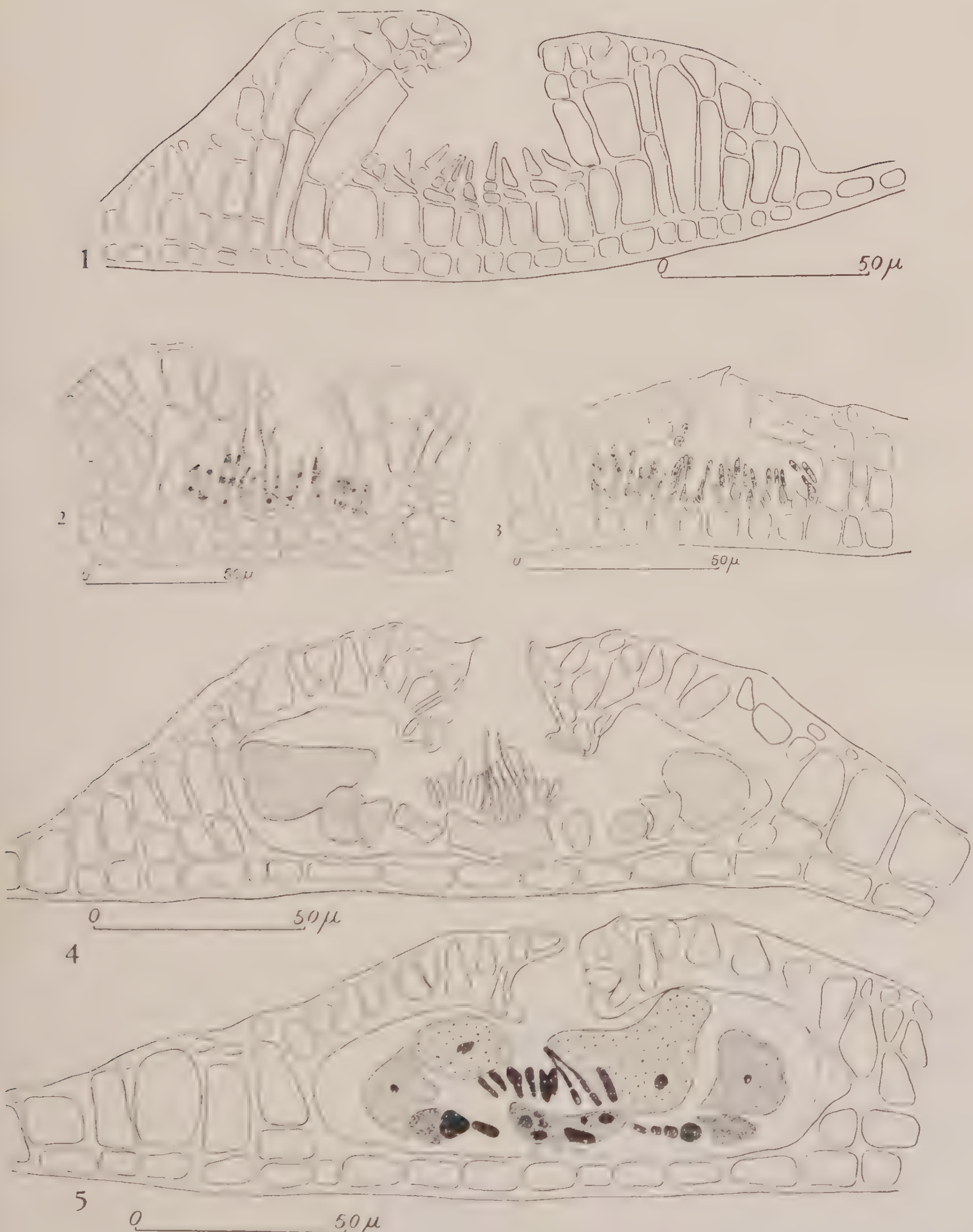
Vertical sections of conceptacles ×650

Fig. 1. Young procarpic conceptacle

Fig. 2. Procarpic conceptacle

Fig. 3. Spermatangial conceptacle

Fig. 4. & 5. Cystocarpic conceptacle; in Fig. 4 is shown the structure of the wall of orifice



T. Masaki & J. Tokida: Studies on the Melobesioideae of Japan. III

STUDIES ON THE BIOLOGY OF THE SEA URCHIN

II. Size at First Maturity and Sexuality of Two Sea Urchins, *Strongylocentrotus nudus* and *S. intermedius*

Akira FUJI

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In a previous paper (Fuji, 1960) it has already been mentioned that most specimens of *S. intermedius* under 20 mm and of *S. nudus* under 30 mm in test diameter possess neuter gonads. This assessment has been concluded from arbitrary samples, but it is not based upon samples with variation of test diameter covering the whole range from large animals to small ones. Consequently, it may be highly necessary to make careful determinations on the biological minimum size, which is an important item of basic information for understanding the reproductive biology of these sea urchins.

To test the validity of this assessment, observations were made on the sexual composition and on the size at first maturity of these animals.

The writer wishes to thank Prof. T. Tamura of the Faculty of Fisheries, Hokkaido University, for his invaluable advice during the course of the present investigation and for his kindness in reading the original manuscript. To Mr. Y. Ogawa and Mr. T. Awakura, the writer expresses his sincere gratitude for their kind aid in collection of materials used.

Material and Method

The materials employed in this investigation consist of two series: (a) To test the sexual composition, regular samples covering the entire size range of the two sea urchins were taken from Muroran and Ishiya during the period from June to September 1958. (b) Other samples for determination of the size at first maturity in their life were arbitrarily selected from the samples of September, that is, at the period of maximum gonad development.

Total number of sea urchins examined was about 2,000 specimens.

These urchins were histologically examined on their gonad tissue and the histological criteria as reported in the previous paper were used for a careful determination of gonad maturity.

Histological techniques employed in this observation are the same as described in the previous paper.

Observation

The data are illustrated in histogram form (Figure 1), the frequencies being the percentages of neuter, male and female urchins for each 5 mm class interval of test

diameter. Figure 1(a) is based on the histological observation for gonads of 644 specimens of *S. intermedius* from Ishiya, (b) on 627 specimens of the same species from Muroran, and (c) on 477 specimens of *S. nudus* from Ishiya. During the period of June – September, the percentage of inactive gonads in the adult specimen is very low, consequently sea urchins with undeveloped gonads are predominantly neuter specimens.

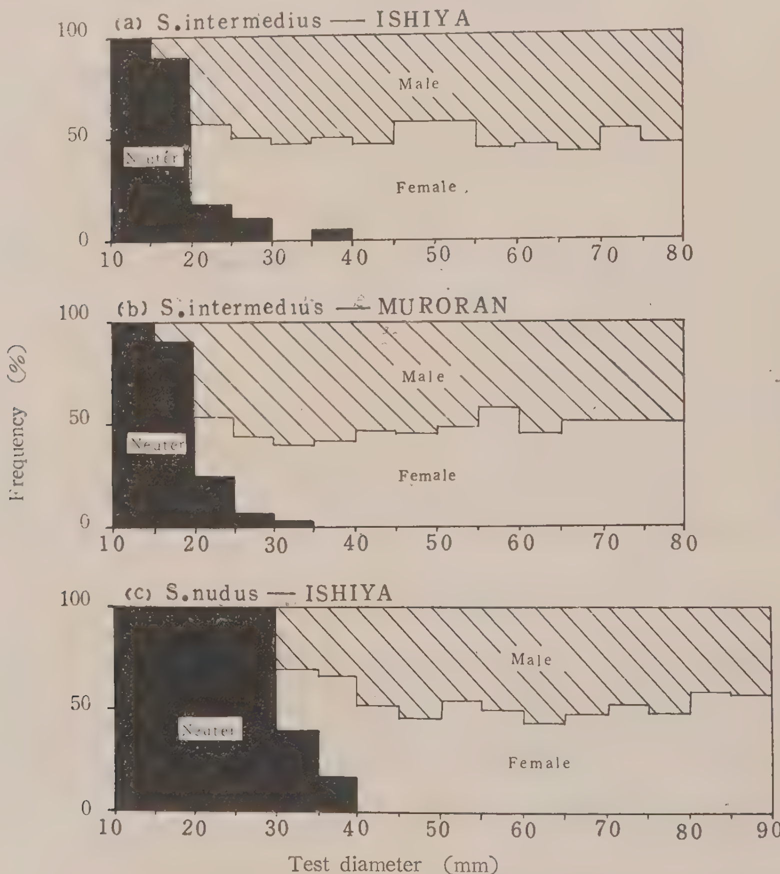


Fig. 1. The percentage frequency of the sexes in various size-groups (based on test diameter)

Figure 1 shows how sex composition varies with size (equatorial diameter, exclusive of spines). In *S. nudus*, almost all specimens of under 30 mm test diameter possess neuter gonads, while in *S. intermedius* neuter specimens were those under 15 mm in test diameter. The two sexes are approximately equal in number in *S. intermedius* having a test diameter over 15 mm; the same is found in *S. nudus* over 30 mm test diameter.

The histograms represented in Figures 2 and 3 are the frequencies in percentage of each gonadal stage for the two species of sea urchins collected in September corresponding to the term of maximum gonad development. In both species the frequency in each gonad

stage shows a considerable fluctuation with variation of test diameter; a significant difference between the two species is found at the size group which represented a high frequency of Stage IV gonad. Specimens of *S. intermedius* of 25 35 mm in test diameter possess mature gonads (Stage IV) in excess of 50 %. On the other hand, in *S. nudus* nearly all specimens of under 35 mm test diameter possess gonads belonging to Stage I, and the animals of Stage IV approach about 50 % when they attain to size between 45 mm and 50 mm equatorial diameter.

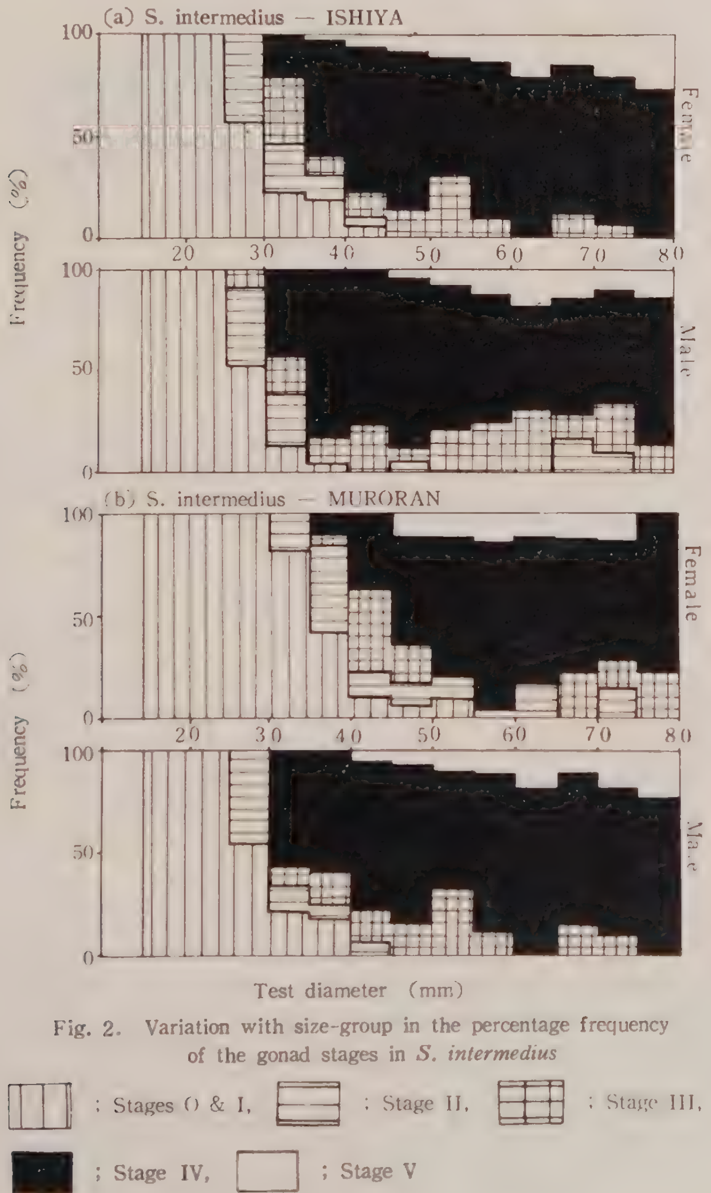


Fig. 2. Variation with size-group in the percentage frequency of the gonad stages in *S. intermedius*

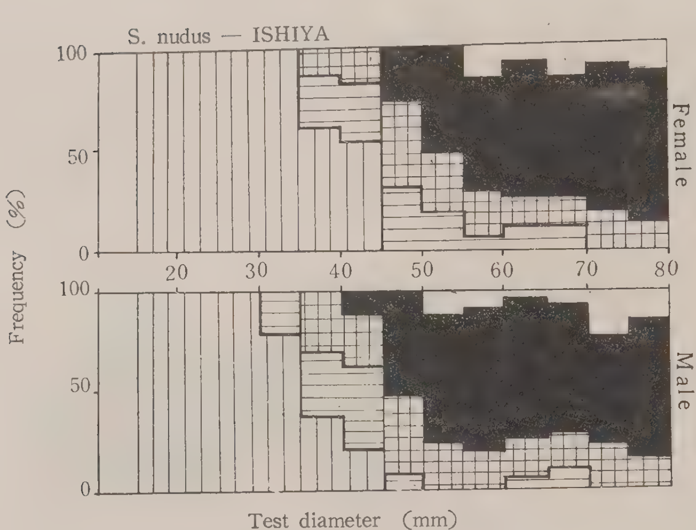


Fig. 3. Variation with size-group in the percentage frequency of the gonad stages in *S. nudus*. Each symbol is the same as those in Fig. 2

In regard to this matter, the most striking difference between male and female urchins is that the small size-group of the male population is preponderant in comparison with the same size-group of female ones in the occupancy of later gonad stages (Stages IV and V). The size-groups of 20–35 mm in *S. intermedius*, like *S. nudus* in size-groups of 30–45 mm, possess a high proportion (ca. 70% at least) of the early developing gonad (Stages I and II) even in September which is the period of maximum gonad development. Accordingly, it is extremely probable that the specimens of these size-groups remain virgin without developing to the later gonad stages till the following spawning season. From such considerations, it is concluded that the size at first maturity covers a test diameter of 30–35 mm in *S. intermedius* and of 40–45 mm in *S. nudus*.

Discussion

Very few statistical reports of hermaphrodite echinoids are available. The record that 20 hermaphrodites are yielded among 10,000 *Strongylocentrotus prupuratus* is the highest incidence so far reported (Boolootian & Moore, 1956). Giard (1900) has suggested that *Echinocardium cordatum* is normally a protandric hermaphrodite. Neefs (1937, 1938, 1952) used two lines of explanation for the seasonal change of sex. One is the presence of hermaphrodite specimens, in which one sexual phase is in perfect condition whilst the other shows degeneration. The other attempt has been made to account for the seasonal variation of sex composition; male urchins preponderate over female in summer

season, and vice versa in winter. The presence of degenerate gonads of two colours in an animal (Neefs, 1952) and of a perfectly functioned hermaphrodite (Shapiro, 1935; Harvey, 1939) may be interpretted as a presumable indication of sex reversal. Since the presence of such a sexual phenomenon is very rare (Shapiro found one individual among 2350 *Arbacia punctulata*), however, it may be an appearance of pathological gonads so called "pseudohermaphrodite".

If echinoids are normally a protandric hermaphrodite, it may be noticed that the great majority of small urchins are male, and that the large ones are preponderantly female. As illustrated in Figure 1, there is no significant change in the sex ratio over almost the entire range of size-groups except as the result of differential mortality and migration into two sexes. In the two sea urchins under consideration in the present investigation, a differential migration and mortality is unlikely between male and female population.

It has been already presumed that the first maturity in their life commences with a test diameter ranging between 30-35 mm in *S. intermedius* and between 40-45 mm in *S. nudus*. Of course, the size dimension is proportional to age; the size range may be regarded as corresponding to the lifetime of the species. However, there is no available data of growth rate in the two sea urchins up to the present time. Consequently, it may not be said with certainty that the difference in the size at first maturity between the two species is a direct indication of different ages. Such questions concerned with age and growth rate of the two species of sea urchins are left as unsolved problems.

Summary

Observations have been made on the biological minimum size of two sea urchins, *S. intermedius* and *S. nudus*, by means of stained section of gonads.

Almost all specimens of *S. intermedius* ranging up to 15 mm test diameter and of *S. nudus* under 30 mm in test diameter were neuter, but in the specimens in excess of these sizes it is possible to differentiate the sexes. The size at first maturity covers a test diameter ranging between 30 and 35 mm in *S. intermedius* and between 40 mm and 45 mm in *S. nudus*.

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STUDIES ON THE BIOLOGY OF THE SEA URCHIN

III. Reproductive Cycle of Two Sea Urchins, *Strongylocentrotus nudus* and *S. intermedius*, in Southern Hokkaido

Akira FUJI

Faculty of Fisheries, Hokkaido University

Investigations on the breeding season have been primarily concerned with relative breeding intensities. The term "breeding season" means the period when the majority of individuals liberate the most gametes; this is preceded by a period of wide spread gonad development and is followed by a period of active appearane of free swimming larvae.

Natural spawning of the sea urchin is not readily visible and there is no certain way of knowing from which local population the free swimming larvae have been derived, as they may be carried away by the action of currents from the area in which they were spawned. It is possible, however, by regular sampling of the population, to determine whether there is a sudden sustained decrease in the percentage of ripe gonads; such an occurrence may be interpretted as a major spawning.

The present paper describes an attempt to follow the cycle of gonad changes which occur throughout the year in each population of two sea urchin species, *Strongylocentrotus nudus* and *S. intermedius*, collected in southern Hokkaido.

The author wishes heartily thank Prof. T. Tamura of Hokkaido University, for his invaluable advice during the course of the present observation and for his kindness in reading the original manuscript. Thanks also are due to Messrs. Y. Ogawa, T. Awakura and S. Sawada for collecting the materials used.

Material and Method

The materials used in this study consist of regular monthly samples from the large group of sea urchins (over 40 mm in test diameter) collected at three localities (viz., Muroran, Ishiya and Shinori) in southern Hokkaido, over the period of June 1956 to May 1959. Total number of animals employed is about 2,500 specimens.

The animals were subjected to anatomical and histological examination detailed in a previous paper (Fuji, 1960).

Result

Annual reproductive cycle of two sea urchins

Observation included two examinations; the first one undertook to determine the seasonal variation of gonad coefficient showing the superficial appearance of gonad maturity, and the other, using histological methods, was carried out to analyse the process of gameto-

genic development and depletion.

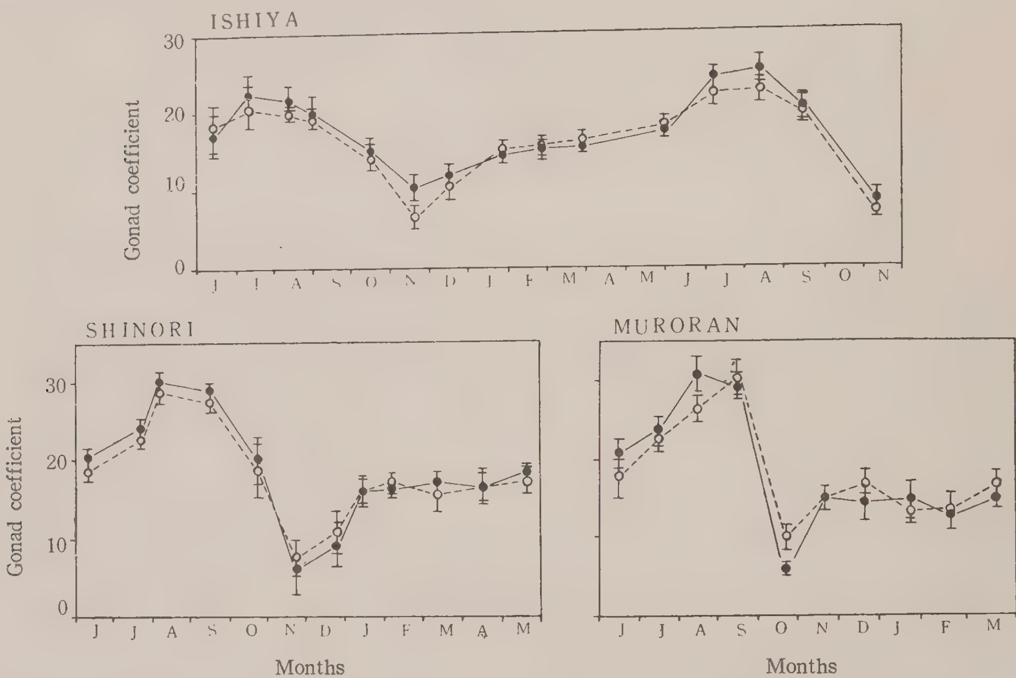


Fig. 1. Cyclic changes of the gonad coefficient in both sexes of *S. intermedius*
Each vertical bar indicates the range of plus or minus in standard error of mean
● ; Male population, ○ ; Female population

Seasonal variation of gonad coefficient. Figures 1 and 2 represent how the gonad coefficient of male and female urchins in both species varies at the different seasons of the year. From the above figures, it is firstly noticed that the average gonad coefficient throughout the year, especially from December to May, indicates a considerable difference between *S. nudus* and *S. intermedius.*

The gonad coefficient of *S. intermedius*, for September, represents a significantly lower value than that for August. The minimum value (ca. 10) of the average gonad coefficient is calculated from the animals collected in November. After this month the value increases slightly, and attains approximately 15 in January or February. Gradual increment of the coefficient value continues steadily during the months between February and May. The value of the gonad coefficient begins to increase strongly from June or July followed by the peak in August. Such seasonal pattern of the gonad coefficient shows a similar trend in the population of sea urchins collected from all localities selected in this observation.

In *S. nudus*, the gonad coefficient increases markedly from June to August when the average coefficient shows a peak value, followed by a comparatively rapid decrease of the

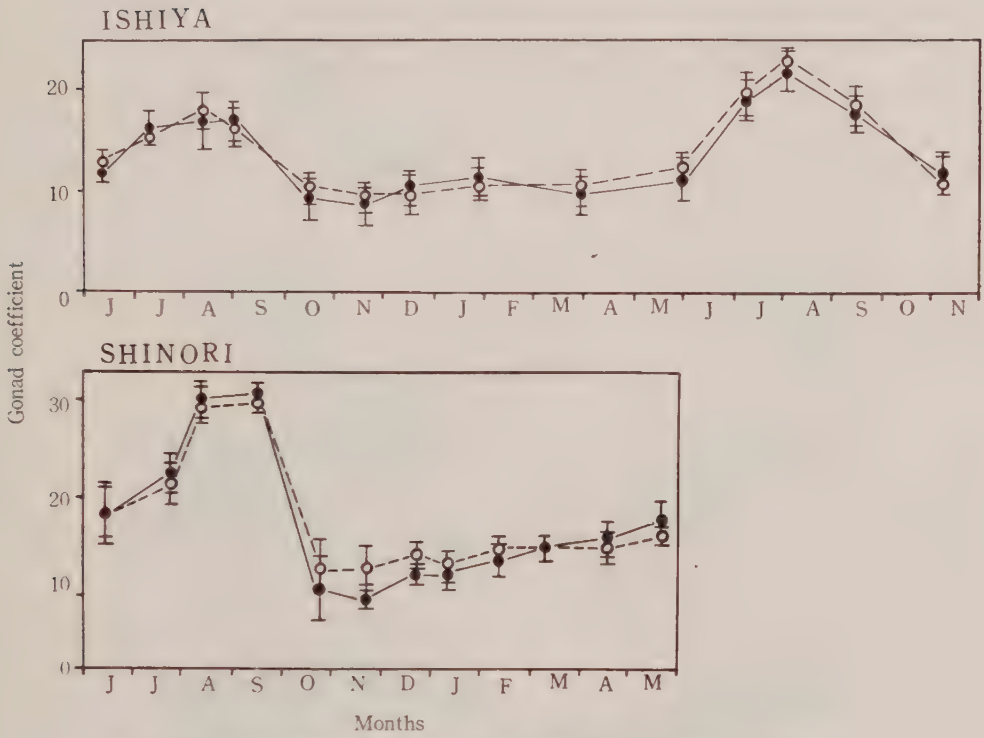


Fig. 2. Cyclic changes of the gonad coefficient in both sexes of *S. nudus*
Each symbol is the same as those in Fig. 1

coefficient during September and October. From December, the gonad coefficient shows a constantly low level until the following April or May, after which the average value indicates about 10.

No significant difference in relative gonad volume between male and female urchins has been recognized in *Strongylocentrotus purpuratus* (Bennett & Giese, 1955). On the other hand, in *Echinocardium cordatum*, Moore (1935) has pointed out that the relative gonad volume of female urchins shows an expansion about twice that of male urchins throughout the entire year. From the data presented (see Figs. 1 and 2), it is clearly indicated that the similarities in seasonal variations of the average gonad coefficient between male and female urchins are more striking than the differences in the two species used in this observation, though the gonad coefficient always fluctuates even in the animals taken at the same period. The most striking difference in the peak value of the gonad coefficient between the 1956 and 1957 seasons is the much higher value for the latter year in both species collected from Ishiya. Moreover, it is noticed that the comparison of the average gonad coefficients of the two urchins collected from three different localities reveals a considerable difference between them. Although the cause of such difference is uncertain,

on the basis of anatomical observation, either descrepancy of the growing process of gametes or the difference of genital crop may be said to account for it.

Gametogenic cycle. As has previously been stated (Fuji, 1960), the gametogenic cycle of the adult sea urchins of the two species under consideration consists of five histological stages of gonad activity. There are; Stage I (Recovering spent), Stage II (Growing), Stage III (Pre-mature), Stage IV (Mature), and Stage V (Spent).

The percentage of each of the five stages of gonadal development and depletion is plotted against the month in Figures 3 and 4. From observation of the graphs it is evident that the cyclic processes of sexual maturity in the two species of urchins run a considerably different course.

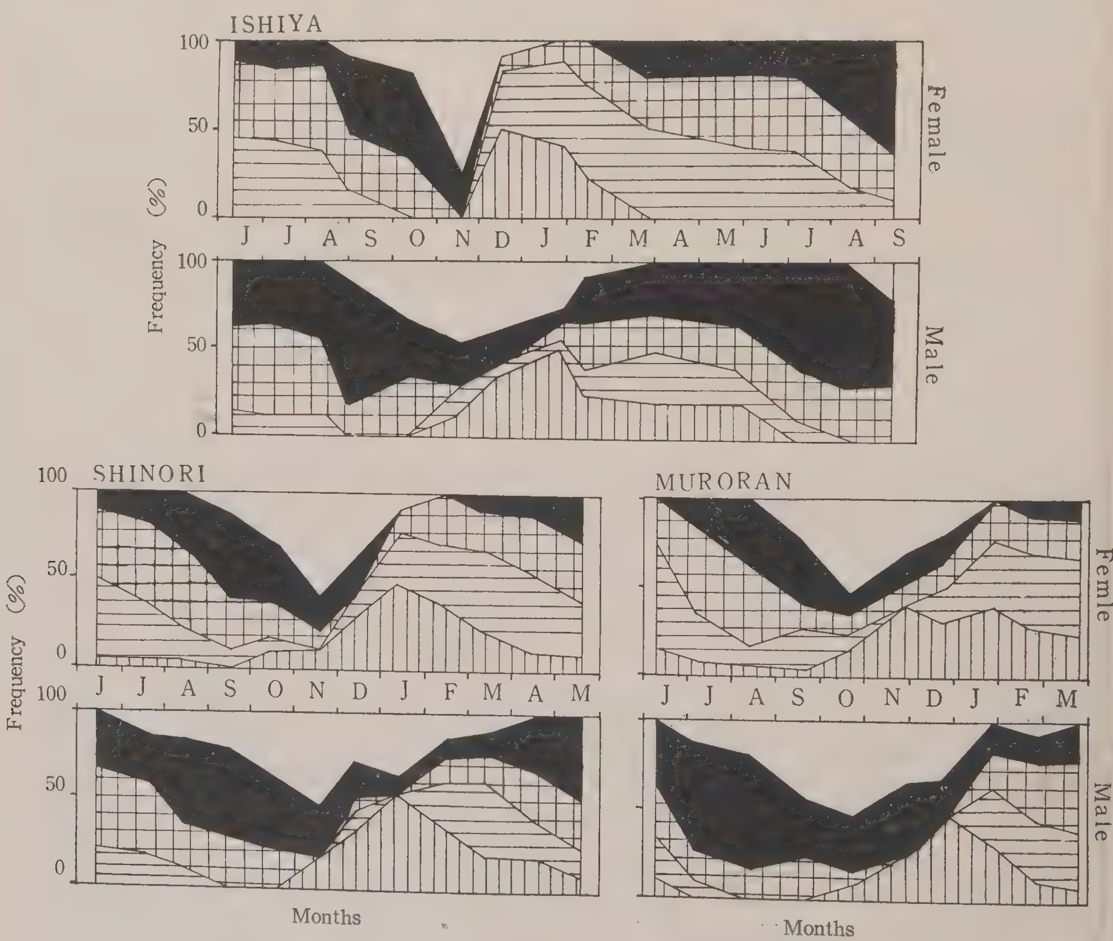
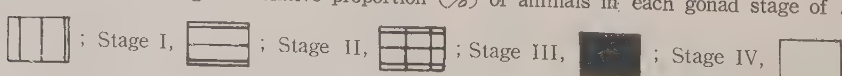


Fig. 3. Diagram showing the relative proportion (%) of animals in each gonad stage of *S. intermedius*



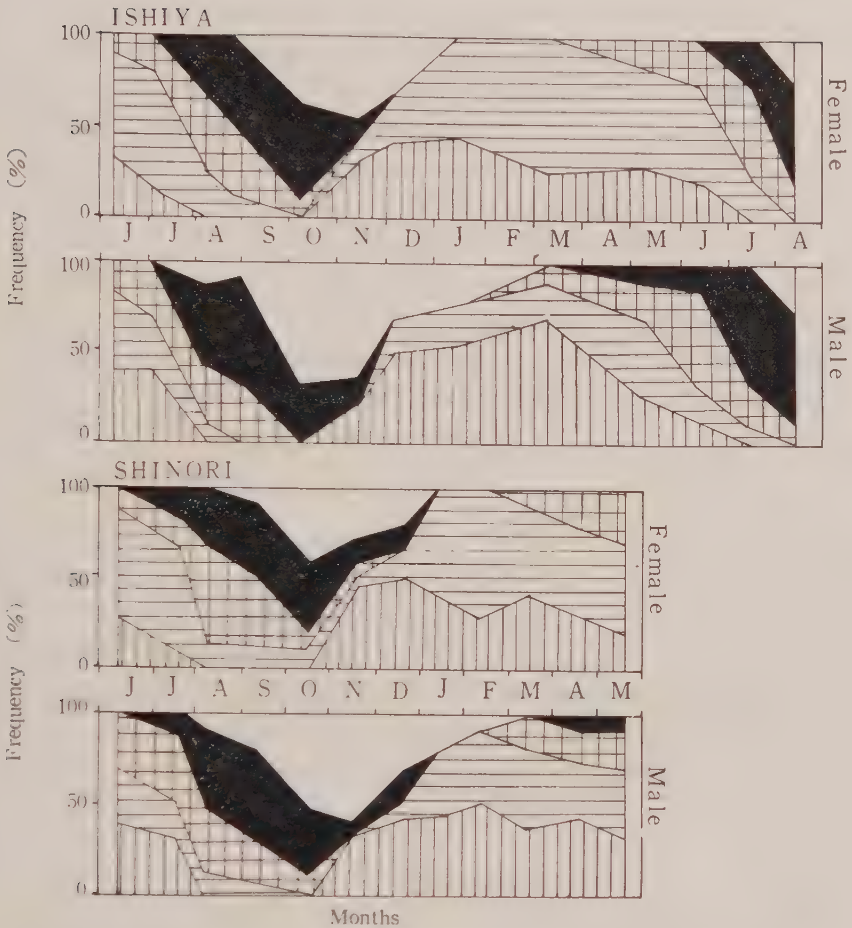


Fig. 4. Diagram showing the relative proportion (%) of animals in each gonad stage of *S. nudus*
All symbols are the same as tho.e in Fig. 3

In August and September, individuals of *S. intermedius* of Stage IV show a comparatively high proportion in their frequency (ca. 50 % in female and ca. 70 % in male), afterward a rapid drop of the frequency lasts until November. From September the spent animals increase vigorously in frequency and attain to a maximum proportion (40 60 %) in November. After this month, simultaneously with the sluggish diminution of the spent individuals, the animals of Stage I (Recovering spent) become conspicuous by their augmentation; nearly half of the animals collected in January possess Stage I gonad. After January, a diminution of Stage I animals is accompanied by a gradual augmentation of Stages II and III animals; such trend is more strongly manifested in the male population than in the others. The mature urchins (Stage IV) provide 20 30 % of the population from March and increase gradually until July and August.

In *S. nudus*, individuals in Stages I and II are found in over 70 % of the entire number of animals examined in June or July, and remainder show Stage III. Afterward the animals of Stages I and II decrease markedly in their frequency, and disappear in October. On the other hand, the animals of Stages III and IV increase rapidly during the period of August – September, and attain to about 40 % among the animals collected in October. Spent individuals (Stage V) are almost not found amongst the animals collected in every month, and their maximum number is limited to about 60 % in October. After November the animals of Stage I increase gradually, and they keep a relatively high proportion (ca. 40 – 50 %) until following April or May, although they are accompanied by individuals which are developing to more advanced condition (viz., Stages II and III) in the successive months. In mid-March the male urchins of Stage IV (Mature) are already found, but their appearance is limited to very low percentages (ca. 6 %). After early June, in both male and female population, the percentage of Stage IV animals increases rapidly with the passage of time.

As regards the seasonal difference of sexual maturity between male and female urchins, it has been pointed out respecting several sea urchins that the male population possesses the mature gametes in an earlier period than the female population (in *Echinus esculentus*, Stott, 1931; Moore, 1934; in *Strongylocentrotus purpuratus* and *S. franciscanus*, Lasker & Giese, 1954; Bennett & Giese, 1955). Such tendency coincides with the conclusion reached from observation of the two sea urchin species employed in this study. Therefore, it may be suggested that male gametes are available virtually throughout the entire year, while female fertilizable gametes are recognized only within some limited term.

Spawning season

The data which indicate that most individuals of the two urchin species are in the shedding condition are diagrammatically given in Figures 3 and 4, which include information on the onset of spawning for several localities in southern Hokkaido.

Sometime between the middle of August and the beginning of September, a comparatively rapid change from the full mature codition to the spent phase takes place. Mean monthly sea temperature in various localities is shown in Figure 5. In comparison between the relative proportion of animals in each gonad stage and the mean monthly sea temperature, spawning of the female urchins seems to have commenced simultaneously at all stations towords the end of September when water temperature is about 17°C, while in male urchins the shedding condition takes place from late July to mid-September.

In view of these items of information, it is concluded that the breeding seasons of the present two sea urchin species in southern Hokkaido are prolonged, extending from early August, and that the majority of the individuals spawn in October or November, although the breeding would be commenced at somewhat different times in various localities.

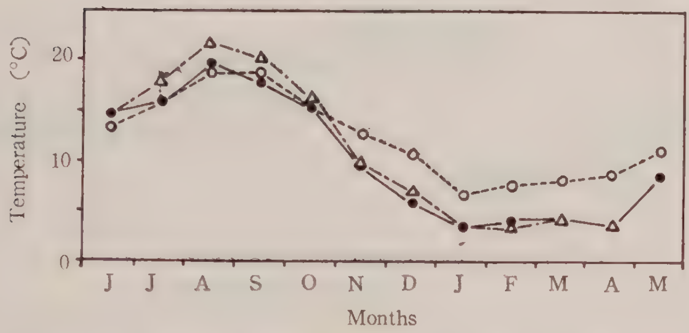


Fig. 5. Seasonal variation of water temperature at various localities in southern Hokkaido ● ; Ishiya, △ ; Muroran, ○ ; Shinori

Spawning was not caused by the attainment of a definite temperature only, but occurred at several temperature in the range of 15–20°C.

Discussion

It has already been mentioned that the increase of the gonad coefficient has been accompanied by increase of reproductive substances (Fuji, 1960). In view of the fact that the seasonal changes of the gonad coefficient are concomitant with the variation in the appearance of each gonad stage, all things considered, the annual reproductive cycle in the present two sea urchin species may be roughly divided into the following three different processes ;

a) June – August : Vigorously developing season.
b) September – November : Spawning season.
c) December – May : Sluggishly developing season.

Figure 6 summarizes schematically the relationship lying between the value of the gonad coefficient and the duration of active appearance of each gonadal stage. During the sluggishly developing season, the pattern of gonadal maturity runs a clearly different course in the two sea urchins used in these observations. Such difference is reasonably considered to result from the sexual phenomena furnished by themselves.

The only observation on the breeding season of *S. intermedius* has been made by Kinoshita (unpublished data) for regular samples collected from Usu along the coast of Volcano Bay in southern Hokkaido. He proposed that there are two main spawnings a year, the one in October to November, the other in April. In another species, *S. pulcherrimus*, Kawana (1938) has reported that the major spawnings take place in the term from January to March. Bennett & Giese (1955) have stated that both *S. franciscanus* and *S. purpuratus* possess a single reproductive cycle. As shown in Figures 3 and 4, the spent individuals reach a high proportion of the total population in October or November. Therefore, so far as the present investigation is concerned, it may be concluded that the

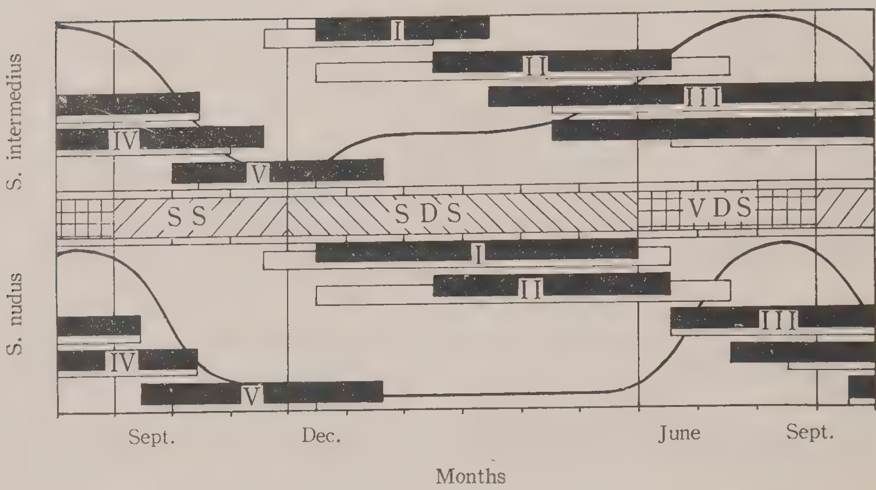


Fig. 6. Schematic illustration of the relation between the seasonal variation of gonad coefficient and the duration of active appearance of each gonad stage [black bar]; Male population, [white bar]; Female population, VDS; Vigorously developing season, SS; Spawning season, SDS; Sluggishly developing season

seasonal trends of the reproductive process of the present two sea urchins show clearly one distinct cycle having the peak in October or November.

As to *Echinus esculentus*, *Strongylocentrotus pulcherrimus*, *S. purpuratus* and *S. franciscanus*, some variations of the relative gonad weight or volume have been shown between the different habitats and years. And it has been presumed that such variations are attributable to the fluctuation of the algal crop furnished in each habitat and year (Moore, 1934; Kawana, 1938; Lasker & Giese, 1954; Bennett & Giese, 1955). On another Echinodermata, *Asterias rubens*, Vevers (1949) has reported that a poorly fed population shows no or little gonad maturity, whereas a well fed population reveals vigorous development of the gonads.

The causative factors involved in the development of the gonads may be numerous. However from the above items of information concerned with the influence of feeding conditions upon gonadal development, it is reasonably presumed that such variation indicated between different localities and years is provoked by seasonal variation of physico-chemical and some biological factors which exert indirect effects upon the urchins by making possible a great production of algae, associated with other many unknown factors. Although none of the presented data deal with the production of algae and other various environmental factors in the course of the present observation, it may be suggested that the influence of feeding condition and of environmental factors upon the gonadal maturity of adult and young urchins provides some unknown problems in various ecological and physiological aspects.

Summary

(1) No significant difference is found between male and female urchins in seasonal variation of the gonad coefficient. The gonad coefficient exhibits a peak value in July or August, and shows minimum value in October or November in both species.

(2) After spawning, the average coefficient value indicates a considerable difference between the two urchins; the average coefficient value of *S. intermedius* increases gradually and shows somewhat higher value than that of *S. nudus* which holds a constantly low level until April.

(3) The gametogenic cycle seems to run a considerably different course between the two species; in *S. intermedius* the duration of active appearance of Stage I (Recorvering spent) animals is relatively short, moreover, the presence of stage IV (Mature) extends over a long term. On the other hand, in *S. nudus*, the predominant presence of the animals with Stage II (Growing) gonad covers the early half-year.

(4) The male population of both species possesses mature gametes for a greater part of the year than does the female population.

(5) The annual reproductive cycle in the two sea urchins may be broadly divided into the following three different processes according to the course of seasonal variation of the gonad coefficient and of gametogenesis:

a) June – August : Vigorously developing season.

b) September – November : Spawning season.

c) December – May : Sluggishly developing season.

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CHEMICAL STUDIES ON MARINE ALGAE

XIV. ON A NEW AMINO ACID, "CHONDRINE", ISOLATED FROM THE RED ALGA *Chondria crassicaulis*

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INTRODUCTION

Few studies have been made about the nitrogenous compounds in marine algae. Recently, however, B. Lindberg detected taurine and N-methyltaurines[1] in several red algae. S. Makisumi isolated arginylglutamine[2] from green alga, *Cladophora* species. Pharmacologically active substances, kainic acid[3] and domoic acid,[4] were isolated from *Digenea simplex* and *Chondria armata* by T. Takemoto and K. Daigo, respectively. The author detected citrulline in the red alga, *Porphyra tenera*[5] and it was isolated from *Rhodogrossum purchrum* and *Chondrus ocellatus*.[6] *l*-Çitrulline and *d*-aspartic acid were also isolated from *Chondria armata*. [7]

The present writers' succeeding investigations on the free amino acids in red algae revealed the existence of an unknown amino acid in *Chondria crassicaulis*, which gave an intense blue colour with ninhydrin after development on paper chromatograms. This substance was isolated from ethanol extract by a technique of displacement chromatography using cationic resin Dowex 50-H,$^+$ and now it has been confirmed to be *l*-1-sulfoxythiazine-3-carboxylic acid. This substance was given the name "chondrine" from the name of this alga, *Chondria crassicaulis*.

EXPERIMENTAL

MATERIAL AND METHOD

The material (*Chondria crassicaulis* Harvey) was collected at Nanaehama, Hakodate on the 10th of Nov. 1958.

The method used for the isolation of this substance was practically the same as for *l*-citrulline from red algae already discribed.[6] All paper chromatograms were run on Tōyō No. 50 filter paper; the solvent used in this experiment were phenol saturated with water, n-butanol acetic acid water (4:2:1 & 4:1:5), collidine lutidine (1:3) saturated with water. As a spraying agent, 0. 2 per cent ninhydrin solution in butanol was used, besides, the following specific tests were employed. A) Isatine solution for proline, B) Ehlrich's reagent[8] for citrulline, C) Plattner's reagent[9] for glycine and 1-sulfoxythiazine-3-carboxylic acid (chondrine).

ISOLATION

To fresh alga (15 kg.), 10 liters of hot water were added and the temperature maintained half an hour at 70° C until solution became muddy. After it became cool, 40 liters of ethanol were slowly added with stirring. Insoluble materials having been filtered off, the extract was concentrated *in vacuo*, decolourized with 100 g of acetic acid treated charcoal.[10] The colourless extract was passed through a large column of Dowex 50–H^+ (2500 ml, 20–50 mesh) to remove inorganic salts. Amino acids fraction was eluted with 0.2 N–NH_4OH. This fraction was applied to the two column system of Dowex 50–H^+ (200 and 60 ml, 100 mesh), washed with 3 liters of water and fractionated with 0.2 N–NH_4OH into 87 fractions 24 ml each. (Fig. 1). The unknown substance eluted in pure state at the top of common amino acids; only a small portion was eluted with aspartic acid. The fraction contaminated with aspartic acid (Fig. 1, Band II) was gathered, adjusted to pH 1.5 by addition of N–HCl, and was applied to a column of Amberlite IR–4B–OH^- (100 ml, 50–100 mesh). The column was washed with 500 ml. of water. The unknown substance was obtained in pure state in a nonabsorbable fraction, whereas aspartic acid remained in the column. Unknown substance fractions so obtained were concentrated *in vacuo*, and crystallized from ethanol. The unknown substance was crystallized 3 times from aqueous ethanol, and dried in a vacuum desiccator over H_2SO_4. Five g of white crystalline material were obtained (Fig. 2A).

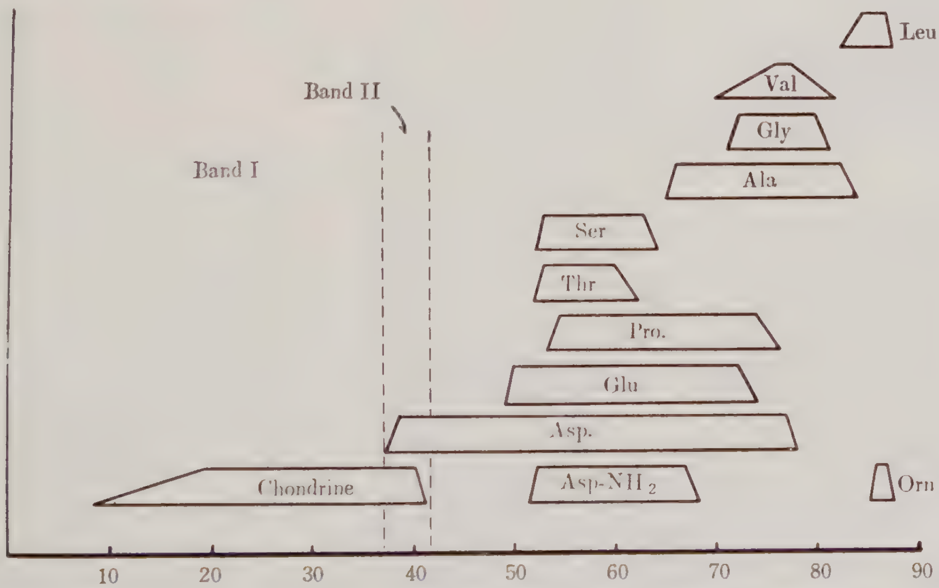


Fig. 1. Primary fractionation of the free amino acids in *Chondria crassicaulis* by a two column system of Dowex 50 X–4 (200 and 60 ml., 100–200 mesh); 0.2 N–NH_4OH was used as a displacement agent

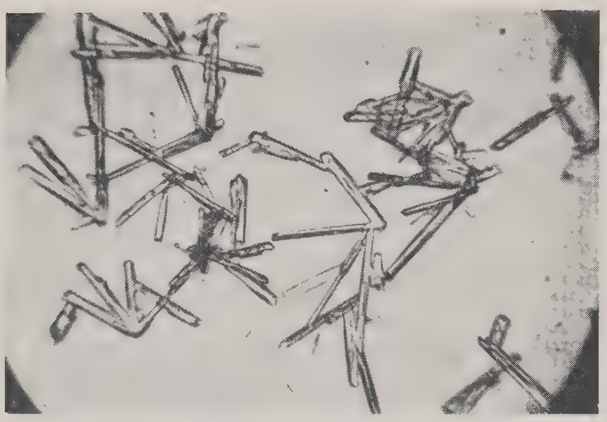


Fig. 2A. Unknown acid from *Chondria crassicaulis*



Fig. 2B. Chondrine Cu salt

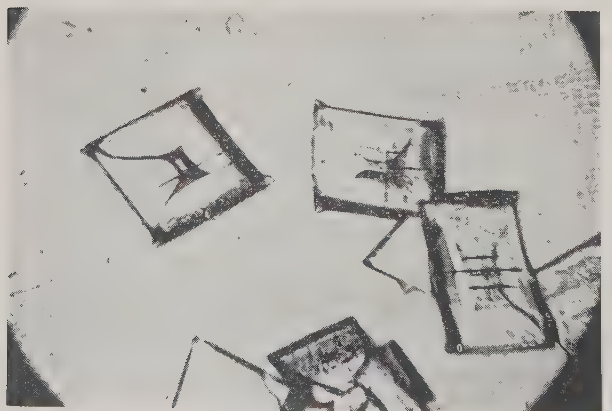


Fig. 2C. DL-N-Ethylalanine (Synthesis)

PROPERTIES OF THIS SUBSTANCE

The most striking property of this substance was its characteristic colour with ninhydrin on paper chromatograms. After development on paper chromatograms, it gave at first cobalt colour with ninhydrin (0.2 per cent in butanol), then deepened to intense blue and at last was changed into brown colour by prolonged heating. Rf values on various solvent systems are shown in Table 1. This substance was unstable for mineral acids and resultant from treatment with 6N-HCl at 100° C for 24 hrs in a sealed tube, the solution gave an intense red colour; paper chromatographically, besides the original substance, a new spot was obtained which no more gave a characteristic colour but a violet colour with ninhydrin (Table 1).

The unknown substance was very soluble in water and virtually insoluble in organic solvents such as ethanol, methanol or aceton. It did not show a definite melting point and slowly degraded above 210° C but it melted with decomposition at 255–257°C in a sealed tube. Molecular weight estimated by cryoscopic method gave 160. Elementary analysis agreed with the formula $C_5H_9O_3NS$.

	C	H	O	N	S
Estimated	36.73%	5.56%	29.44%	8.63%	19.64%
Calculated	36.81	5.52	29.45	8.59	19.63
as $C_5H_9O_3NS$		O ; by difference			

Optical rotation gave values $(\alpha)_D^{16}=+20.91$ (C=2) in water and $(\alpha)_D^{12}=+30.15$ (C=2) in 6N-HCl.

This substance was led to a copper salt. Five hundred mg were dissolved in 10 ml of water and kept for 30 minutes on a boiling water bath with one g of copper carbonate. After an excess of copper carbonate, had been filtered off, copper salt was crystallized from ethanol (Fig. 2B).

	N	Cu
Estimated	6.26%	14.34%
Calculated	6.34	14.38
as $C_{10}H_{22}O_6N_2S_2Cu$		

For the estimation of crystalline water, 200 mg of copper salt was dried *in vacuo* over P_2O_5 at 110°C. Estimated water correspond to 12.20 per cent of the salt so that this copper salt gave a formula $(C_5H_8O_3NS)_2Cu3H_2O$. It decomposed at 171°C in a sealed tube.

Thus this substance gave a copper salt, but gave no nitrogen when treated by Van Slyke method, so that a secondary amine group was assumed. But the reaction with sodium nitroprusside[illegible] for the secondary amine group was negative. On the other hand, it gave a strong red colour with quinhydron[illegible] characteristic for the secondary amine group. This substance (about 3 mg) was dissolved in 2 ml of 50 per cent ethanol. To the solution, 1 ml of 2.5 per cent quinhydron solution in methanol was added with stirring. Immediately, strong red colour was developed. When proline and hydroxyproline were tested for under the same condition, reactions seemed slightly insenstive because colour production occurred one or two minutes after the test solution had been added. This substance also showed positive with Plattner's reagent. After development on paper chromatograms, it was sprayed with 2 per cent p-nitrobenzoylchloride in benzene, next with pyridine; a bright brown spot immediately developed but faded in a little while.

For the estimation of carboxylic acid, phenolphthalein being used as an indicator, this substance was titrated with sodium hydroxide solution.

Unknown subst	N/50-NaOH titrated (f=0,998)	-COOH Estimated	Calcd. as $C_5H_9O_3NS$
19.0 mg	5.85 ml	27.66 %	27.61 %
16.6 mg	5,10 ml	27.60 %	

Negative result being obtained with diazo or ferric chloride reaction for the hydroxyl group, oxygen, other than carboxylic acid, might exist as a sulfoxide. But all attempts to obtain the reduction product with bisulfites were unsuccessful. This strong binding of oxygen, with the addition of a rather large specific optical rotation of this substance,

led to the conclusion that this substance might exist with a sulfur linkage. A reduced product was obtained by treatment with hydriodic acid, which could be converted to the original substance with hydrogen peroxide solution.

REDUCTION WITH HYDRIODIC ACID

Two g of this substance were dissolved in 5 ml of water and 15 g of hydriodic acid was added. The solution was boiled for 10 hours in an oil bath under a reflux. After removal of excess of hydriodic acid under a reduced pressure, 20 ml of $N-NH_4OH$ was added. Excess of ammonia was again removed under a reduced pressure. The residue, dissolved in water, was applied to a column of Dowex 50-H^+ (40 ml), and washed with 500 ml of water. Amino acid was displaced with 0.2 $N-NH_4OH$ and was crystallized from ethanol (yeild, 1.1 g).

This reduction product no longer gave a characteristic colour as an original substance, but gave a violet colour with ninhydrin (Table 1). Also it gave a bright brown colour with Plattler's reagent. This reduction product was practically the same as that obtained by treatment with 6N-HCl (Table 1).

The reduction product sublimated slowly above 190°C but in a sealed tube it was melted with decomposition at 262-263°C. Elementary analysis gave the formula $C_5H_9O_2NS$

	C	H	O	N	S
Estimated	40.79%	6.18%	21.73%	9.54%	21.76%
Calculated	40.82	6.12	21.77	9.52	21.77
as $C_5H_9O_2NS$		O ; by difference			

Optical rotation gave a value $(\alpha)_D^{13} = -52.94$ (C=2) in water and $(\alpha)_D^{13} = -26.38$ (C=2) in 6N-HCl.

CONVERTION TO THE ORIGINAL SUBSTANCE

One g of this reduction product was dissolved in 6 ml of N-HCl. To the solution, 0.75 g of 30 per cent hydrogen peroxide solution was added. It was kept in a boiling water bath for an hour under a reflux and then overnight at room temperature. The solution was neutralized by addition of equimolar sodium hydroxide solution, desalted by being passed through a column of Dowex 50-H^+. Paper chromatographically it completely agreed with the original substance not only as to its Rf values but also as to it characteristic colour. Small unoxidized product being still encountered, the solution was refractionated on a column of Dowex 50-H^+, 0.2N-NH_4OH being used as a displacement agent. Eight hundred mg. of crystalline were obtained. Melting point gave 255-256°C and its copper salt melted at 171°C (sealed tube). Estimated N gave 8.60% (Calculated N, 8.59%). Optical rotation gave a value $(\alpha)_D^{11} = +19.97$ (C=1) in water.

REDUCTION WITH RANEY NICKEL

Two g of crystalline were dissolved in 40 ml of 50 per cent ethanol. The solution was boiled for 8 hours under a reflux with about 5 g of raney Ni prepared by the method of R. Mozingo.[13] Raney Ni was filtered off and it was washed with about 100 ml of dilute

sodium hydroxide solution. The filtrate was neutralized with hydrochloric acid, desalted by passage through a column of Dowex 50-H^+. The main reduction product, which gave a positive result with Plattner's reagent (Table 1, A) and small spot (Table 1, B), were observed on paper chromatograms. The latter, a negative result being obtained with Plattner's reagent, seemed to be an α amino acid. The solution was co chromatographed with known amino acids and it was found that alanine agreed well with it on various paper chromatograms.

To obtain the main reduction product with raney Ni, the solution was applied to a two column system of Dowex 50-H^+ (10 and 3 ml). Amino acids were displaced with 0.1 N-NH_4OH into 17 fractions, 5 ml each (Fig. 3). Fractions eluted with alanine were again fractionated with a small column of Dowex 50 and from the pure solution so obtained, 800 mg of the main reduction product was crystallized from ethanol. Elementary analysis gave the formula $C_5H_{11}O_2N$.

	C	H	O	N
Estimated	51.33%	9.35%	27.44%	11.88%
Calculated	51.26	9.46	27.32	11.95
as $C_5H_{11}O_2N$		O; by difference		

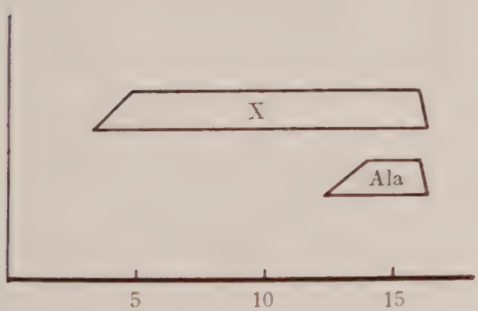


Fig. 3. Fractionation of reduced products with catalyst raney Ni. Two column system of Dowex 50 X-4. (10 and 3 ml. 100-200 mesh) was used and reduction products were fractionated into 17 fractions 5 ml each.

Specific optical rotation gave a value $(\alpha)_D^{15} = +10.41$ (C=1) in water. The reduction product melted with decomposition at 282–285°C in a sealed tube.

The main reduction product gave a copper salt and also gave a positive result with Plattner's reagent so that it seemed to be a N-alkyl-amino acid. On the other hand, by a reduction with raney Ni, a small amount of alanine was detected, so that the structure of N ethylalanine was assumed. These considerations were confirmed by a synthesis of N-ethylalanine. An attempt to obtain an optically active form of this amino acid from *l* alanine by a modified method of *d* methyl alanine described by E. Fischer[14] did not give a good result. But the use of Strecker's method[15] was successful.

SYNTHESIS OF DL-N-ETHYLALANINE

Cold ethylamine hydrochloride solution (42 g in 80 ml of water) was combined with potassium cyanate solution (30 g in 60 ml of water). To the solution, freshly prepared acetaldehyde (20 g) was dropwise added with stirring in an ice bath keeping the reaction mixture below 5°C. When acetaldehyde had been added, the reaction mixture was kept 3

days after transference into a brown bottle tightly closed. The reaction mixture was then transferred to a beaker, and was boiled with 50 ml of concentrated hydrochloric acid until concentration became difficult because of a precipitation of potassium chloride. After the solution became cool, insoluble salt was filtered off. The insoluble salt was washed with 100 ml of cold concentrated hydrochloric acid. After concentration of the solution *in vacuo* to dryness, the residue was extracted with 100 ml of methanol on a hot water bath under a reflux. Insoluble ammonium chloride was fitered off, and it was washed with methanol. The extract was concentrated *in vacuo* to a dryness, the residue was dissolved in 100 ml of water. To the solution excess of zinc carbonate was added to remove the chloride ion in the solution. The precipitate having been filtered off, it was washed with ice cold water. Filtrate was boiled for 30 minutes to remove ammonia and then saturated with hydrogen sulfide. Precipitate was filtered off. The solution was clarified with charcoal, concentrated *in vacuo*, crystallized from ethanol. Twelve g of white crystalline was obtained. (42 % theoretical, Fig. 2 C)

	C	H	O	N
Estimated	51.27%	9.37%	27.40%	11.96%
Calculated	51.26	9.46	27.32	11.95

as $C_5H_{11}O_2N$ O; by difference

Synthetically obtained N-ethylalanine melted with decomposition at 282-284°C in a sealed tube. As shown in Table 1, this substance was found to quite agree with the main reduction product with raney Ni on the various paper chromatograms.

Table 1. Rf values of chondrine and its degraded products in various solvents.

Substance	Solvent I	Solvent II	Solvent III	Solvent IV
Original	0.69	0.27	0.17	0.61
Reduction with HI	0.80	0.53	0.37	0.68
Treatment with 6N-HCl	0.69 0.80	0.27 0.53	0.17 0.37	0.61 0.68
Reduction with raney Ni (A)	0.88	0.62	0.42	0.44
(B)	0.58	0.44	0.26	0.52
Oxidation with H_2O_2 (C)	0.39	0.17	0.15	0.59
(D)		0.29	0.19	0.46
(E)	0.58	0.44	0.26	0.52
(F)	0.63	0.63	0.41	
Alanine	0.58	0.44	0.26	0.52
DL-N-ethylalanine (Synthesis)	0.88	0.62	0.42	0.44
Taurine	0.39	0.17	0.15	0.59

Solvent I, Phenol saturated with water. Solvent II, n-Butanol acetic acid water (4:2:1). Solvent III, n-Butanol acetic acid water (4:1:5) upper phase. Solvent IV, Colidine lutidine (1:3) saturated with water.

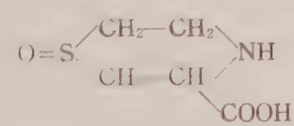
OXIDATION WITH HYDROGEN PEROXIDE SOLUTION

The unknown substance (160 mg) was dissolved in 1 ml of N-HCl and 1 g of 30 per cent hydrogen peroxide solution was added. The solution was boiled in an oil bath for 3 hours under a reflux. It was then passed through a small column of Dowex 2-OH^-, and developed on paper chromatograms. Three spots were detected in the solvent system of phenol water and colidine lutidine, whereas four spots were detected in the solvent system of n butanol (Table 1, C F). Spots C and E quite agreed with taurine and alanine respectively when the material was co-chromatographed with them. Furthermore, spot C gave a positive result with zenphel reaction,[16] so that spot C was confirmed to be taurine. On the other spots (D or F) no further investigation was performed.

CONCLUSION

For the isolation of this unknown substance, it was very efficient to use an ion exchange resin Dowex 50-H^+ and in one fractionation, an almost pure sample of the substance was obtained. Impurity could be removed by a refractionation on a small column of Dowex 50-H^+, but the use of Amberlite IR-4B was more efficient. Its characteristic colour with ninhydrin and a specific test with Plattner's reagent on paper chromatograms made it easy to identify this amino acid.

The unknown substance gave an empirical formula $C_5H_9O_3NS$. Negative results were obtained for the hydroxyl group, so that oxygen other than carboxylic acid was considered to exist as a sulfoxide. But the substance was stable against reduction with bisulfites and the reduction product was obtained by a treatment with hydriodic acid. From this strong binding of oxygen, a sulfur linkage amino acid was assumed. Rather large specific optical rotations of this substance and the reduction product also confirmed this assumption. Because this reduction product could be recovered to the original substance when the reduction product was oxidized with hydrogen peroxide solution, the existance of sulfoxide group was evident. On the other hand by a reduction with raney Ni, *l*-N-ethylalanine as the main reduction product was obtained, which was synthetically confirmed.



(I)

Farthermore, by oxidation with hydrogen peroxide solution the material gave, paper chromatographically, taurine, and other unknown spots. From these results, the substance isolated was thus confirmed to be *l* 1 sulfoxythiazine 3 carboxylic acid (structure I). This substance was given the name "chondrine" from the name of the alga, *Chondria crassicaulis* from which it was derived.

Though thiazine-3,5-carboxylic acid was synthetically obtained by R. D. Coghill,[17]

an amino acid which included the thiazine ring is thought not to have been detected in nature before.

T. Takemoto[3] isolated kainic acid from *Digenea simplex* and K. Daigo[4,7] isolated domoic acid, *l*-citrulline and *d*-aspartic acid from *Chondria armata*. It is very interesting that these algae belong to the same family, Rhodomelacease, especially, interesting that *Chondria armata* and *Chondria crassicaulis* belong to the same species. Though there is such a close relation between the two, not a trace of domoic acid was detected in the present experiment.

This new amino acid has not been detected in other algae so for examined.

SUMMARY

A new amino acid "chondrine" was isolated from the red alga *Chondria crassicaulis* by means of ion exchange resin Dowex 50-H^+. It was identified as *l*-1-sulfoxythiazine-3-carboxylic acid.

The authors acknowledge with hearty thanks assistance received from the Takeda Pharmaceutical Co. in respect to elementary analysis made during the investigation. They are also grateful to Dr. Y. Izumi, Ōsaka University for his valuable suggestions.

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水産無脊椎動物筋肉中の酸可溶核酸成分　I

貝類筋肉中の酸可溶核酸成分に及ぼす貯蔵温度の影響　1

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I. Acid-soluble Nucleotides in Muscle of Marine Invertebrates.

Effects of storing temperature upon the content of muscular nucleotides of some seashells (I)

Ken-ichi ARAI

Abstract

Fresh muscles of seashells were stored at −5°C for 15 days or at 20°C for 70 hours. During these periods of storage the changes in the amounts of nucleotides, nucleosides and purine bases have been followed by means of ion exchange chromatography. The results may be summarized as follows:

(1) Values of ATP, ADP, and AMP (μM/g muscle wet wt.) were 3.10, 1.05, and 0.55 for the fresh foot muscle of surf clam (Spisula sachalinensis), 6.40, 1.20, and 0.60 for the fresh ligament muscle of scallop (Pecten yessoensis), respectively.

(2) During the cold storage of both sorts of fresh muscles at −5°C there was a decomposition of ATP accompanying the formation of ADP and AMP which furthermore were converted to inosine. A small amount of hypoxanthine was found only in the muscle of surf clam. Generally, the changes in the muscle of surf clam were faster than in scallop.

(3) When the samples of both fresh muscles were held at 20°C scarcely any changes could be observed for about 20 hours from the start. After that the changes in the amounts of nucleotides occurred rapidly.

[illegible]，哺乳動物に関しては，骨格筋，脳，肝臓その他種々の組織中の酸可溶核酸成分の定量及び種々条件下における変化に就て[illegible]報告がなされている。

水産動物筋肉に就ては，N.F.Jones による Codling に関する報告及び斎藤等の鯉に関する報告，各種冷凍魚に関するもの，又はカツオ魚に関する[illegible]報告があるが，しかし水産無脊椎動物筋肉中の酸可溶核酸成分の定量及び変化に関する研究は，すでに M.Florkin 等が指摘しているように比較生化学的見地から非常に興味ある問題をもっているにも拘らず，斎藤及び著者の軟体動物のイカに関する報告の他には余り例を見ない。斎藤及び著者はさきに，イカ筋肉には家兎筋肉に劣らないATPが含まれておりながら，死後は他動物筋肉に比して非常に急速な分解が見られる事及び筋肉内に強い活性を示す Adenosine deaminase が認められた事，その分解経路は主経路として ATP→ADP→AMP→Adenosine→Inosine→Hypoxanthine を示し，従来，哺乳類，魚類等の脊椎動物筋肉において発見されている AMP deaminase による IMP の生成は，殆んど無い事をのべた。

本研究はイカと同じ軟体動物に属する[illegible]生活史の異なる貝類の中，ホッキ，ホタテの筋肉を試料として，

[illegible]，費用の一部は斎藤恒行教授担当の農林省農林漁業応用試験研究補助金によつて支弁した。ここに感謝する。

酸可溶核酸成分の定量及び変化を検討した結果に就て報ずる。

実 験 方 法

試料は同じ二枚貝に属するホッキ及びホタテを用いた。いずれも函館近海で漁獲されたもので，水揚後数時間以内で必ず生存状態のものを選んだ。麻酔剤又は液体空気等を使用せず，先ず貝柱（閉殻筋肉）の一端を切り離し刺激を与えないように迅速に採取した。次いでこれをシャーレに入れて一定温度に放置し，時間経過に従つて一定量を採肉，常法どおり過塩素酸で磨砕，抽出を行い分析に供した。酸可溶核酸成分の分析はイオン交換樹脂法により， paper chromatography を併用した[10]。又， nucleotides の酸分解による構成 purin base の確認に就てもすでに述べた。

実 験 結 果

新鮮なホッキ筋肉及び20°Cにおいて28時間放置した同筋肉中の酸可溶核酸成分のイオン交換樹脂からの溶出ヒストグラフを第1図及び第2図に示した。第1図及び第2図において，A区分は270mμに吸収極大を示し，イオン交換樹脂（Amberlite IRA-400）に再クロマトしても吸着されず，又，paper chromatography によつても明確なスポットを得る事が出来ない未確認の物質である。この区分は時間経過による規則的変化が無く，一連の核酸成分の動的変化とは無関係のようである。B区分は新鮮筋肉には無く，250mμに吸収極大を示し， 250/260＝1.4〜1.5の値を与え，更に paper chromatography によつて Inosine と Hypoxanthine からなる事実を確かめた。C区分は AMP, D区分はADP，E区分はATP であつて260mμにいずれも吸収極大を示し， 250/260＝0.80〜0.85であり，酸分解による構成塩基の確認では， Adenine が主成分で未確認の微かなスポットを一ケ含んでいる。この微量成分に関する検討は今後行うつもりでいる。

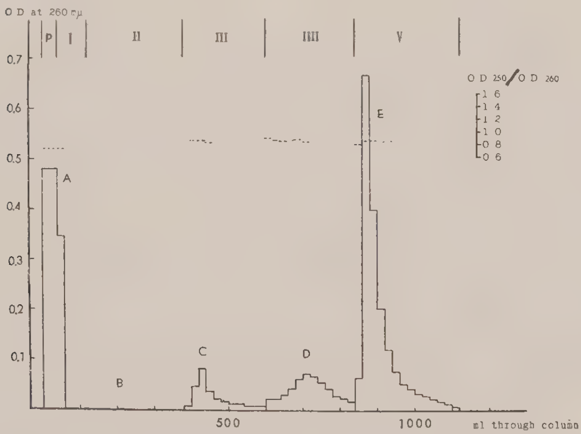


Fig. 1. Separation of acid-soluble nucleotides of the fresh foot muscle (surf clam) by ion exchanger
Exchanger : Amberlite IRA-400, Flow speed : 1ml. /min.
Solvent: P…Passed solution, I…0.01 M NH_4Cl-0.1 M NH_4OH, II…0.01 M NH_4Cl, III…0.005 M HCl, IV…0.02 M NaCl-0.01 M HCl, V…0.2 M NaCl-0.01 M HCl
A…Unknown compound, B…Inosine + Hypoxanthine, C…AMP, D…ADP, E…ATP

尚又，ホタテ筋肉についても全く同じような溶出ヒストグラフが得られた。

A）ホッキ貝（うばがい）Spisula sachalinensis

ホッキは可食肉質部の中いわゆる斧足とよばれる部分で，外観上均質となつている部分に就て分析を行つた。

先ず-5°C 附近で筋肉を緩慢凍結する場合の変化を第3図に示した。 第3図によると，新鮮なホッキ筋肉には主成分として ATP が 3.10 μmole/g mus. wet wt. 存在し，ADP，AMPはそれぞれ1.05, 0.55 μmole/g mus. wet wt. であるが，凍結貯蔵が15日間に及ぶと，ATP は比較的急速に分解し，ADP 次いで AMP

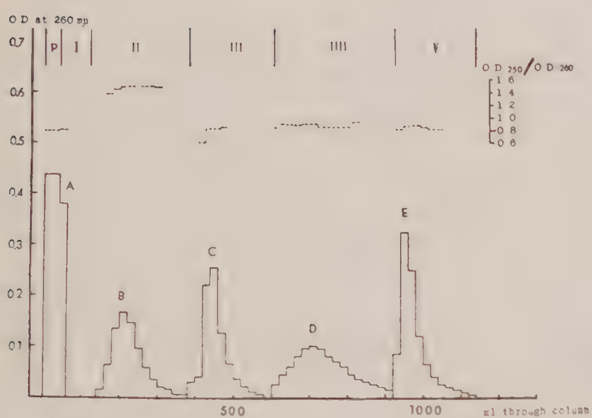


Fig. 2. Separation of acid-soluble nucleotides of the foot muscle (surf clam) stored at 20°C. for 28 hours by ion exchanger

Optical densities of B fraction were shown at 250mμ. The experimental conditions are the same as those in Fig. 1

が分解生成物として生成，増加し次いで減少，更に少し遅れて Inosine とその10%程度の Hypoxanthine が最終的に生成，蓄積してくることがわかつた。ATPの分解は1日後で30%，3日後で16%に至ることから，他の貝類筋肉（アワビ，アカガイ）[11] に比べてかなり速いと云える。

次に筋肉の放置温度が20〜22°Cの場合の同様成分の変化を検討し，その結果を第4図に示した。第4図によると，新鮮な筋肉に存在したATP，ADP，AMPは約15時間に至る迄殆んど変化が無く（別に行つた予備的な実験結果から認めた），このような放置温度において切りとつた同筋肉の生存限界を暗示するもののようである。15時間以後になると ATP は急速に分解し，これに対応的に Inosine

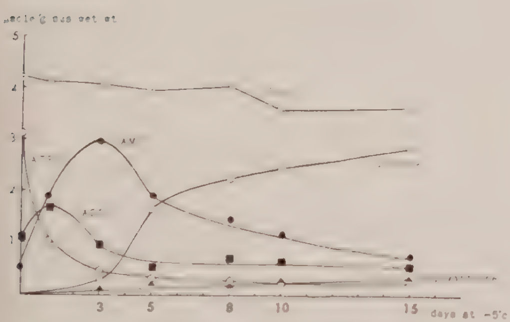


Fig. 3. Changes in adenine nucleotides of the foot muscle of surf clam caused by slow freezing

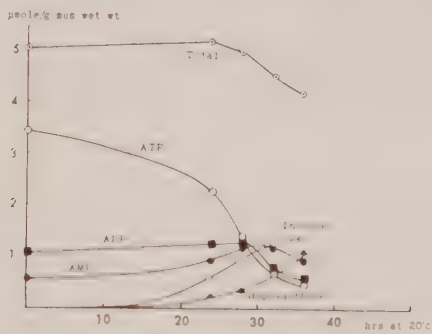


Fig. 4. Changes in adenine nucleotides of the foot muscle of surf clam at room temperature

次いで Hypoxanthine が生成，蓄積してくる。AMPは初め少し増加するがすぐ減少し，ADPは殆んど変化しないが後に減少する傾向を示す。36時間以後に若干の腐敗臭を発生した。なお放置時間が28時間以上になるとき，酸可溶核酸成分の総量が減少する傾向が見られるが，その原因については明らかでない。これは紫外部吸収を示さない低分子化合物や最終産物にまで分解するのか，または筋肉が失ドリップの中に核酸成分の一部を溶出することによるものか不明である。

B）ホタテ貝　Pecten yessoensis

ホタテはその可食肉質部の大部分を占めている貝柱筋肉を選び試料とした。

先ず-5°C附近で筋肉を緩慢凍結する場合の変化を検討した結果を第5図に示した。第5図によると，新鮮なホタテ貝柱筋肉には ATPが 6.40μmole/g mus. wet wt. 主成分として存在し，ADP，AMP はそれぞれ 1.20，0.60 μmole/g mus. wet wt. であり，ATP 量及び総量（ATP+ADP+AMP）はホッキ筋肉に比べて，略2倍に近い値で非常に多い。この ATP は凍結貯蔵で3時間後で45%，10時間後で13%に減少し，ホッキの場合より遅い分解速度を示した。ATPの分解に従つて，先ず3時間頃迄が ADP が徐々に生成次いで

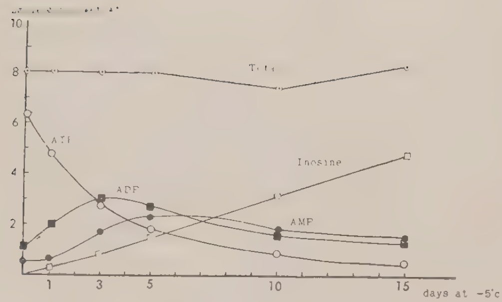


Fig. 5. Changes in adenine nucleotides of the ligament muscle of scallop caused by slow freezing

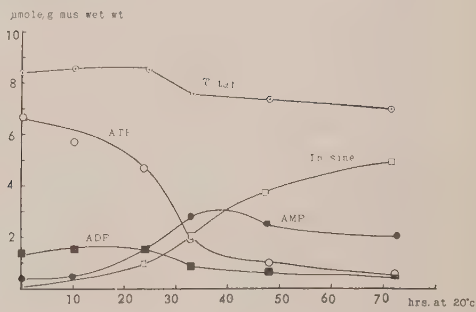


Fig. 6. Changes in adenine nucleotides of the ligament muscle of scallop at room temperature

減少し，更にAMPが5～7時間迄同じく徐々に増加更に減少の傾向を示し，対応的にInosineが凍結日数に比例的に生成，蓄積している。なお，Hypoxanthineはpaper chromatographyによつては，全く認められず，生成しないようである。この点ホッキ筋肉と甚だ異つている。

次に筋肉を18～20°Cに放置した場合の同様成分の変化を検討してその結果を第6図に示した。第6図によると，ホッキ筋肉の場合ほど顕著ではないが，ATPの分解は初め非常に緩慢であつて24時間迄に29%が分解をうけ残りの71%が保持されているが，24時間以後は急速の分解をうけて33時間後には27%になる。ADPには大きな変化は無いが15時間前後に多少蓄積の傾向を示し，以後徐々に減少次いでAMPが38～40時間に至る迄増加し，更に緩慢に減少している。それに対応して最終的反応生成物として，Inosineが24時間迄は緩慢に以後急速に増加，蓄積の傾向を示した。Hypoxanthineは緩慢凍結の場合と同様に全く見出す事が出来ず，この化合物の生成に関連する酵素系の欠除を思わせる。

考　　察

一般に筋肉中に存するATPを中心とする酸可溶核酸成分の死後における変化は，筋肉の生理状態例えば疲労度その他人間の外観的な観察による判断では困難ないくつかの要因によつて，影響をうけるものと考えられる。従つて試料となる水産動物が漁獲された季節，及び漁獲後研究者が入手する迄の処理状態，及び放置温度，時間等々の条件に就ても検討すべき余地がが残されている。本実験の試料はすべて函館近海産のもので，水揚後数時間内の必ず生存しているもののみを選んだ。しかし，そのような注意にも拘らず変化の過程に試料の個体差があらわれた。即ちホッキ筋肉において，ATPの分解にともなうADP及びAMPの最大生成を示す時間は，第3図の1及び3時間後に対して，別の実験では3及び5時間となつているなどその一例である。ただし特別な一成分のみの変化速度に差異があらわれることはなくて，各成分の変化の相対的な関連そのものに変りはなかつた。

生存している二枚貝から貝柱筋肉を切りとる場合には，不本意な刺激を与える事になるのでATPの分解を促がす可能性があり，好ましくない。この場合，麻酔剤又は液体空気を使用する事が考えられるが，その点について得た結果は後報にゆずる。本実験では標準となつている新鮮筋肉でのATPの全酸可溶核酸成分に対する割合は，ホッキ筋肉では70%でやや少く，ホタテ貝柱筋肉では79%であつてイカについて斎藤及び著者が得た80～90%に近い。筋肉内のATP絶対量に就ては，試料が採取される季節によつて多少の変動があるけれども，上記の百分率には殆んど変りなく生存時の筋肉中のATP量にかなり近似しているように考えられる。なお，ホッキ筋肉を20°C近辺に放置する場合，約15時間に至る迄ATPの分解が殆んど進まず新鮮状態のまま保持される現象は，程度の差こそあれホタテの場合にも認められ，アカガイ，アワビにおいても認められる貝類独得の共通現象である。筋肉を他のすべての組織から切り離して放置する場合，魚類（コ

イニジマス[12]），イカ[13]，甲殻類（カニ，エビ）[14]では，ただちに急速なATPの分解が観察されるにも拘らず貝類に限り変化が認められなく，ATPの再生産が長時間持続されている様な状態を保持する事は，貝類一般の生命保持力の旺盛さを裏づける事にもなるので興味深い。

ATP の酵素分解生成物としては，ADP，AMP，Inosine が認められまたホッキの場合のみ少量の Hypoxanthine が見出された。これらの結果は，斎藤及び著者の報じた同じ軟体動物のイカ筋肉に甚だ良く類似している。ただしイカの場合は大量の Hypoxanthine を生成する点だけが異つている。なお，哺乳類[15]，魚類[1,5]両筋肉において必ず見出される IMP は全く認めることが出来なかつた。著者は更にホッキ，ホタテ両筋肉ホモジェネートを酵素液として，Adenosine，AMP，IMP等の基質に対する酵素反応を検討した結果，両筋肉共に強力な Adenosine deaminase を含み AMP deaminase は殆んど存在しない事を知つたが，詳細は後報にゆずる。また二枚貝に属するアカガイとアワビ筋肉に就て酸可溶核酸成分の変化を検討した結果，ATP の酵素分解物として ADP，AMP の他に少量の Adenine を見出し，本報告に示されたホッキ，ホタテに就て知られた変化様式と明らかに異る一群の貝類を認めたが，次号にこれを報告する。

要　　　約

ホッキ筋肉及びホタテ貝柱筋肉を−5℃及び20℃に保つて，筋肉中の酸可溶核酸成分の変化を検討し次の結果を得た。

1） 新鮮なホッキ筋肉中の酸可溶核酸成分は，ATP，ADP，AMP が筋肉 1 g当り3.10，1.05，0.55μmole であり，ホタテ貝柱筋肉中にはそれぞれ6.40，1.20，0.60 μmole であつて，ATP が主成分であつた。

2） −5℃で凍結貯蔵すると，両筋肉とも ATP が減少し，ADP，AMP が続いて増加次いで減少し，更に Inosine が生成し，ホッキの場合のみ少量の Hypoxanthine の生成が認められた。一般にホッキの方がホタテの場合より変化が早い。

3） 20℃で貯蔵するときは，両筋肉とも初めの中，ATP が減少せず新鮮な状態を保持するが，一定時間後に急速に減少し，各成分の変化が早く起る。

本研究は斎藤恒行教授の指導の下に行つた。ここに記して深く感謝する。

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MARINE ALGAE EPIPHYTIC ON LAMINARIALES PLANTS

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Contents

Introduction

Since the time of Tobler's classical work entitled "Epiphyten der Laminarien," published in 1909, many phycologists have accumulated numerous data about the epiphytic marine algae growing on or in the thallus of Laminariales plants. However, no one has ever published to date a list of those epiphytes, so far as the writer is aware. The list here presented has been prepared by consulting 63 publications of previous investigators and by adding certain new data obtained during a research on the organisms growing on the Laminariales plants that was carried out by the writer in collaboration with one of his students (Tokida & Yama, 1960).

Epiphytism of seaweeds is in certain cases a fixed character of a species but in others merely an occasional phenomenon occurring in a species only by chance. Many seaweeds which usually grow on rocks can also attach epiphytically to other seaweeds if conditions are favourable. So it may seem meaningless to enumerate the epiphytes of Laminariales plants. As a matter of fact, however, there is always a definite tendency in the quality and quantity of epiphytic seaweeds growing on a certain species of Laminariales at a given locality and in a given season. Far distance migration of a seaweed may be a matter of possibility if the seaweed grows epiphytically on a detached and drifting thallus of a kelp such as *Nereocystis luetkeana*. Epiphytic organisms growing more or less markedly on some economically important kelps may draw the attention of fishermen. A list of epiphytic marine algae such as the present one will be of some use in providing certain items of information for a study of these and related problems.

The present list comprises 126 genera and 285 species of seaweeds that have been reported to be epiphytic on the Laminariales plants belonging to 22 genera. These epiphytes

include 2 genera and 3 species of Cyanophyceae, 16 genera and 22 species of Chlorophyceae, 37 genera and 116 species of Phaeophyceae, and 71 genera and 144 species of Rhodophyceae. They are all attaching directly to, or growing in the tissue of, the surface of the host frond except only two endozoic species, viz., *Endodictyon infestans* GRAN (No. 47) and *Acrochaetium infestans* HOWE et HOYT (No. 154). The former grows in the body of a Bryozoa, *Membranipora* sp., attached to the old blade of *Laminaria*, and the latter in stolons and hydrocaules of a Hydrozoa, *Obelia geniculata*, attached to the frond of *Laminaria*.

Certain relations between the hosts and the epiphytes are summarized in Tables I and II. Among the 22 genera of Laminariales, *Laminaria* stands first in the number of epiphytic algal genera which count 106 in total. Next comes *Alaria* whose epiphytes are 27 in number of genera. Then follows *Chorda* with 21 epiphytic genera. The giant kelps, *Macrocystis* and *Nereocystis*, show comparatively few epiphytes contrary to one's quite natural expectation, having been reported to furnish substrata to 4 and 10 genera of epiphytic algae respectively. From the view point of discovery of new epiphytic species and forms, however, *Nereocystis luetkeana* is most remarkable among the hosts as it has been reported by Setchell & Gardner (1922, 1925) to be the host on which they discovered and described many new algae including 9 species of *Compsonema*, 2 *Myrionema* and one *Streblonema*, not to speak of varieties and forms.

As learnt from the present list, a host plant is often mentioned in the literature with its full specific name but sometimes only with its generic name. As for the genus *Laminaria*, 16 specific names can be picked up from the list. Of those species, *L. hyperborea** stands first in the number of epiphytes which comprise 50 genera and 66 species; next come *L. digitata* and *L. saccharina* whose epiphytes comprise 36 genera with 61 species and 24 genera with 29 species respectively. For the remaining 13 species of *Laminaria*, their respective epiphytes are recorded to be only one to six species in number.

In view of the literature cited, it may be said that the present list gives the general aspect of the epiphytic algae of the Laminariales plants known from the noteworthy regions of the world if not a complete enumeration of all the epiphytes ever reported.

**Laminaria hyperborea* in this paper includes *Laminaria Cloustonii* according to the conception of Du Rietz, G. E. (Nomenclature of *Laminaria digitata* (HUDS.) LAMOUR. and *Laminaria hyperborea* (GUNN.) FOSLIE. *Sv. Bot. Tidskr.* 47 (1), 15-23. 1953).

Table I. Generic names of the hosts and the epiphytes that are treated in the present paper and the relation betwee them

Epiphyte \ Host	Chorda	Agarum	Alaria	Arthrothamnus	Costaria	Dictyoneurum	Ecklonia	Eckloniopsis	Egregia	Eisenia	Hedophyllum	Laminaria	Lessoniopsis	Macrocystis	Nereocystis	Pleurophycus	Pleuropterum	Postelsia	Pterygophora	Saccorhiza	Thalassiophyllum	Undaria
CYANOPHYCEAE																						
Xenococcus (2)*	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Calothrix (1)	+	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
CHLOROPHYCEAE																						
Ulothrix (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Acrochaete (1)	+	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Bolbocoleon (1)	+	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Pseudodictyon (1)	...	...	...	...	...	+	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Pseudopringsheimia (2)	...	...	...	...	...	...	...	...	+	...	...	+	...	...	...	...	...	...	...	...	...	...
Ulvella (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Acrosiphonia (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Chaetomorpha (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Cladophora (2)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Rhizoclonium (1)	+	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Spongomorpha (3)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	+	...
Ulva (2)	...	...	...	...	...	...	...	...	+	...	...	+	...	...	...	...	...	...	...	...	...	...
Monostroma (2)	...	...	+	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Codiolum (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Derbesia (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Struvea (1)	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
PHAEOPHYCEAE																						
Ectocarpus (21)	+	...	+	...	...	...	...	...	+	...	...	+	+	...	+	...	...	...	...	+	+	...
Endodictyon (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Entonema (2)	+	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Laminariocolax (1)	...	...	+	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Phaeostroma (2)	+	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Pylaiella (3)	...	...	+	...	...	...	...	...	...	...	...	...	...	...	...	+	...	+	...	+	...	...
Streblonema (9)	...	...	+	...	...	...	...	...	...	...	+	+	...	...	+	...	...	...	...	...	...	...
Ralfsia (2)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Sphacelaria (4)	+	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Chaetopteris (1)	...	...	+	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Halopteris (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Acrothrix (2)	+	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Chordaria (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Papenfussiella (1)	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...	...	...
Microcoryne (1)	+	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

(*Continue*)

(Continued)

Host / Epiphyte	Chorda	Agarum	Alaria	Arthrothamnus	Costaria	Dictyoneurum	Ecklonia	Eckloniopsis	Egregia	Eisenia	Hedophyllum	Laminaria	Lessoniopsis	Macrocystis	Nereocystis	Pleurophycus	Pleuropterum	Postelsia	Pterygophora	Saccorhiza	Thalassiophyllum	Undaria
Elachista (1)												+										
Leptonema (1)			+									+								+		
Ascocyclus (5)			+									+										
Chilonema (1)												+										
Clathrodiscus (1)																				+		
Compsonema (11)									+		+	+			+							
Hecatonema (8)			+									+			+							
Myrionema (14)					+				+			+		+	+							
Symphyocarpus (1)			+									+										
Coilodesme (1)												+										
Dictyosiphon (2)	+											+										
Giraudia (1)	+											+										
Myriotrichia (1)	+																					
Asperococcus (3)												+										
Desmotrichum (1)	+											+										
Halorhipis (1)									+													
Litosiphon (5)	+		+									+								+		
Rhadinocladia (1)	+																					
Isthmoplea (1)												+										
Desmarestia (1)												+										
Tilopteris (1)												+										
Laminaria (2)			+									+										
RHODOPHYCEAE																						
Goniotrichum(1)	+																					
Erythrocladia (1)												+										
Erythrotrichia (2)												+			+							
Porphyropsis (1)												+										
Porphyra (5)			+									+		+	+				+	+		
Porphyrella (1)									+			+										
Acrochaetium (10)		+					+		+			+							+	+		
Audouinella (1)												+										
Chantransia (1)												+										
Kylinia (1)			+																			
Rhodochorton (7)			+									+										
Dumontia (1)												+										
Cruoriella (1)												+										

(Continue)

(*Continued*)

Epiphyte \ Host	Chorda	Agarum	Alaria	Arthrothamnus	Costaria	Dictyoneurum	Ecklonia	Eckloniopsis	Egregia	Eisenia	Hedophyllum	Laminaria	Lessoniopsis	Macrocystis	Nereocystis	Pleurophycus	Pleuropterum	Postelsia	Pterygophora	Saccorhiza	Thalassiophyllum	Undaria
Peyssonelia (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Rhododermis (2)	...	...	+	+	...	...	...	...	...	...	...	+	...	...	...	...	...	...	+	...	+	...
Cruoria (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Petrocelis (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Callophyllis (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Cirrulicarpus (1)	...	...	+	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	+	...
Euthora (2)	...	+	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Kallymenia (1)	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	+	...	...	+	...	...	...
Clathromorphum (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Corallina (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Dermatolithon (5)	...	...	+	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Epilithon (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Heteroderma (1)	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...	...	...
Lithophyllum (1)	...	...	...	...	...	...	+	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Lithothamnium (5)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Melobesia (3)	...	...	...	...	...	...	+	...	...	...	...	+	...	...	...	...	...	...	...	...	...	+
Phymatolithon (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Chondrus (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Phyllophora (2)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Plocamium (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Cystoclonium (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Rhodophyllis (2)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Bonnemaisonia (2)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Gastroclonium (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Lomentaria (3)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Botryocladia (1)	...	...	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...
Rhodymenia (2)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	+	...	...	...	...	...	...	...
Antithamnion (8)	...	...	...	...	...	...	...	...	...	...	...	+	...	+	+	...	...	...	...	...	...	...
Callithamnion (3)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Campylaephora (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Ceramium (7)	+	...	+	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Microcladia (1)	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...	...	...	...
Neoptilota (1)	...	...	+	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	+	...
Pleonosporium (1)	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	+	...
Plumaria (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Ptilota (3)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	+	...

(*Continue*)

(*Continued*)

Epiphyte \ Host	Chorda	Agarum	Alaria	Arthrothamnus	Costaria	Dictyoneurum	Ecklonia	Eckloniopsis	Egregia	Eisenia	Hedophyllum	Laminaria	Lessoniopsis	Macrocystis	Nereocystis	Pleurophycus	Pleuropterum	Postelsia	Pterygophora	Saccorhiza	Thalassiophyllum	Undaria
Ptilothamnion (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Spermothamnion (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Acrosorium (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Apoglossum (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Delesseria (2)	...	...	+	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	+	...
Hymenena (1)	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...
Hypoglossum (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Membranoptera (4)	...	...	+	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	+	...	...	...
Pantoneura (3)	...	...	+	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Phycodrys (2)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Yendonia (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Heterosiphonia (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Amplisiphonia (1)	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...
Brongniartella (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Chondria (1)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Laurencia (2)	+	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Odonthalia (1)	...	...	+	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Placophora (2)	...	...	...	...	...	...	+	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Polysiphonia (9)	+	...	+	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	+	...	...
Pterosiphonia (1)	...	...	+	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Rhodomela (2)	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...
Taenioma (1)	...	...	...	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...

* Numeral in parentheses is the number of species enumerated in the genus concerned.

List of Epiphytes

CYANOPHYCEAE

PLEUROCAPSALES

PLEUROCAPSACEAE

1. **Xenococcus laminariae** J. FELDM.

   J. Feldmann (1954): On *Laminaria hyperborea* (blade).

2. **X. crouanii** J. FELDM.

   J. Feldmann (1954): On *Laminaria ochroleuca* (blade).

Table II. Number of epiphyte genera reported to have been found on each of the 22 host genera

Host	Number of Epiphytic algal genera				
	Cyano.	Chloro.	Phaeo.	Rhodo.	Total
Laminaria	2	13	30	61	106
Alaria	0	1	11	15	27
Chorda	1	3	12	5	21
Nereocystis	0	0	5	5	10
Egregia	0	2	4	3	9
Thalassiophyllum	0	1	1	6	8
Saccorhiza	0	0	5	3	8
Pterygophora	0	0	0	7	7
Macrocystis	0	0	1	3	4
Ecklonia	0	0	0	4	4
Agarum	0	0	0	2	2
Eisenia	0	0	1	1	2
Hedophyllum	0	0	2	0	2
Pleurophycus	0	0	1	1	2
Arthrothamnus	0	0	0	1	1
Costaria	0	0	1	0	1
Dictyoneurum	0	1	0	0	1
Eckloniopsis	0	1	0	0	1
Lessoniopsis	0	0	1	0	1
Pleuropterum	0	0	0	1	1
Postelsia	0	0	1	0	1
Undaria	0	0	0	1	1

OSCILLATORIALES

OSCILLATORIACEAE

3. **Calothrix confervicola** (ROTH) AG.

Printz (1926): On *Chorda filum*, *Laminaria* (blade).

CHLOROPHYCEAE

ULOTRICHALES

ULOTRICHACEAE

4. **Ulothrix flacca** (DILLW.) THUR.

Jónsson (1901-3): On *Laminaria*.

CHAETOPHORACEAE

5. **Acrochaete repens** PRINGSH.

Jónsson (1901-3), Kylin (1910), Waern (1952): On *Chorda filum*.

Taylor (1937, 1957): On *Chorda* (cortex), *Laminaria* (cortex).

6. **Bolbocoleon piliferum** PRINGSH.
   Kylin (1910): On *Chorda filum*.
   Setchell & Gardner (1920), Kylin (1949): On *Chorda*.
7. **Pseudodictyon geniculatum** GARDN.
   Setchell & Gardner (1920): On *Laminaria sinclairii*.
   G. M. Smith (1944): On *Dictyoneurum californicum* (cortex).
8. **Pseudopringsheimia apiculata** SETCH. et GARDN.
   Setchell & Gardner (1920), G. M. Smith (1944): On *Egregia menziesii*.
9. **P. confluens** (ROSENV.) WILLE
   Kylin (1910, as *P. penetrans* KYLIN), Levring (1937): On *Laminaria hyperborea*.
10. **Ulvella fucicola** ROSENV.
   Jónsson (1901–3): On *Laminaria digitata* (stipe).

CLADOPHORACEAE

11. **Acrosiphonia spinescens** (KÜTZ.) KJELLM.
   Printz (1926): On *Laminaria* (old blade).
12. **Chaetomorpha melagonium** (WEB. et MOHR.) KÜTZ.
   Kylin (1910): On *Laminaria hyperborea* (stipe).
13. **Cladophora rupestris** (L.) KÜTZ.
   Kylin (1910): On *Laminaria hyperborea*.
14. **Cladophora** sp.
   Sundene (1953): On *Laminaria saccharina*.
15. **Rhizoclonium kochianum** KÜTZ.
   Sundene (1953): On *Chorda filum*.
16. **Spongomorpha arcta** (DILLW.) KÜTZ.
   Printz (1926): On *Laminaria*.
17. **S. duriuscula** COLL. var. **tenuis** YAMADA
   Yamada (1935): On *Thalassiophyllum clathrus* (blade).
18. **Spongomorpha** sp.
   Jónsson (1901–3): On *Laminaria hyperborea*.

ULVALES

ULVACEAE

19. **Ulva angusta** SETCH. et GARDN.
   G. M. Smith (1944): On *Egregia laevigata*.
20. **U. pertusa** KJELLM.
   Tokida & Yama (1960): On *Laminaria japonica* and *L. religiosa* (blade).

MONOSTROMACEAE

21. **Monostroma lactuca** (L.) J. AG.
   Printz (1926): On *Laminaria* and *Alaria*.

22. M. undulatum WITTR.

Jónsson (1901–3): On *Laminaria* (stipe).

CHLOROCOCCALES

CHLOROCHYTRIACEAE

23. Codiolum petrocelidis KUCK.

Jónsson (1901–3): On *Laminaria hyperborea* (stipe).

SIPHONALES

DERBESIACEAE

24. Derbesia marina (LYNGB.) KJELLM.

Printz (1926): On *Laminaria hyperborea.*

Levring (1937): On *Laminaria* (stipe).

VALONIACEAE

25. Struvea delicatula KÜTZ.

Segawa (1938): On *Eckloniopsis radicosa* (blade).

PHAEOPHYCEAE

ECTOCARPALES

ECTOCARPACEAE

26. Ectocarpus confervoides (ROTH) LE JOL.

Printz (1926): On *Laminaria* (stipe, blade).

f. **typica** KJELLM.

Rosenvinge & Lund (1941): On *Chorda filum, Laminaria digitata.*

f. **dasycarpa** (KUCK.) ROSENV.

Rosenvinge & Lund (1941) : On *Laminaria* (stipe).

f. **penicillata** (AG.) KJELLM.

Rosenvinge & Lund (1941) : On *Chorda filum.*

f. **pygmaea** (ARESCH.) ROSENV.

Rosenvinge & Lund (1941) : On *Laminaria digitata* (stipe, blade).

G. M. Smith (1944, as var. *pygmaeus* (ARESCH.) KJELLM.) : On Laminariales.

f. **siliculosa** (AG.) KJELLM.

Rosenvinge & Lund (1941) : On *Chorda filum, Laminaria.*

27. E. cylindricus SAUND.

Setchell & Gardner (1925) : On *Egregia menziesii.*

G. M. Smith (1944) : On Laminariales, *Egregia menziesii.*

28. E. dimorphus SILVA

G. M. Smith (1944, as *E. variabilis* (SAUND.) G. M. SMITH.): On *Laminaria setchellii, Lessoniopsis littoralis.*

Silva (1957), Scagel (1957) : On Laminariales.

29. E. draparnaldioides (CR.) KJELLM.

Kjellman (1883), Levring (1937) : On *Laminaria digitata.*

Kylin (1910): On *Laminaria digitata, L. hyperborea.*

Printz (1926) : On *Laminaria* (blade).

Rosenvinge & Lund (1941): On *Laminaria digitata, L. saccharina.*

J. Feldmann (1954) : On *Laminaria* (blade), *Saccorhiza polyschides* (blade).

30. E. **fasciculatus** (GRIFF.) HARV.

Jónsson (1901-3) : On *Laminaria digitata* f. *stenophylla*, *Alaria esculenta* (stipe,blade).

Kylin (1910) : On *Alaria.*

Printz (1926) : On *Laminaria digitata.*

Levring (1937) : On *Laminaria.*

Taylor (1937, 1957): On Laminariaceae.

Rosenvinge & Lund (1941) : On *Laminaria digitata, Chorda filum.*

J. Feldmann (1954) : On *Chrda filum.*

Jaasund (1957) : On *Laminaria digitata, L. hyperborea.*

31. E. **fructuosus** SETCH. et GARDN.

G. M. Smith (1944) : On *Nereocystis luetkeana.*

32. E. **fusiformis** NAGAI

Yamada & Tanaka (1944) : On *Laminaria* sp. (blade).

Nagai (1941) : On *Thalassiophyllum clathrus* (stipe).

33. E. **granulosoides** SETCH. et GARDN.

G. M. Smith (1944) : On *Nereocystis luetkeana.*

34. E. **granulosus** (SMITH) AG.

Rosenvinge & Lund (1941) : On *Laminaria digitata.*

J. Feldmann (1954, as *Giffordia granulosa* (SMITH) HAMEL): On *Laminaria saccharina.*

35. E. **hiemalis** CROUAN

Printz (1926) : On *Laminaria hyperborea* (blade).

36. E. **hincksiae** HARV.

Jónsson (1901-3) : On *Laminaria hyperborea.*

Printz (1926) : On *Laminaria* (old blade).

Levring (1937) : On *Saccorhiza bulbosa.*

J. Feldmann (1954, as *Giffordia hincksiae* (HARV.) HAMEL): On Laminariales (blade), particularly on *Saccorhiza polyschides.*

37. E. **ovatus** KJELLM.

Printz (1926) : On *Laminaria* (stipe).

38. E. **oviger** HARV.

Setchell & Gardner (1925), G. M. Smith (1944) : On *Nereocystis luetkeana.*

Scagel (1957) : On *Nereocystis* (stipe).

39. E. **penicillatus** (AG.) KJELLM.

Jónsson (1901-3) : On *Laminaria digitata, Alaria esculenta.*

Printz (1926) : On *Laminaria.*

40. **E. sandrianus** ZANARD.

Rosenvinge & Lund (1941) : On *Laminaria digitata.*

41. **E. secundus** KÜTZ.

J. Feldmann (1954, as *Giffordia secunda* (KÜTZ.) BATT.) : On *Saccorhiza polyschides* (apex of leathery segment of blade), *Laminaria digitata.*

42. **E. siliculosus** (HUDS.) LYNGB.

Printz (1926) : On *Chorda filum, Laminaria digitata, L. saccharina.*

Sundene (1953) : On *Chorda tomentosa.*

J. Feldmann (1954, as *E. siliculosus* (DILLW.) LYNGB.) : On *Laminaria saccharina* (blade).

43. **E. terminalis** KÜTZ.

Setchell & Gardner (1925) : On *Alaria fistulosa.*

Printz (1926) : On *Laminaria digitata* (stipe).

44. **E. tomentosus** (Huds.) LYNGB.

Printz (1926) : On *Laminaria digitata* f. *stenophylla* (blade).

45. **E. yezoensis** YAMADA et TANAKA

Yamada & Tanaka (1944) : On *Laminaria* sp.

46. **Ectocarpus** sp.

Tokida & Yama (1960) : On *Laminaria ochotensis.*

47. **Endodictyon infestans** GRAN

Printz (1926) : Endozoic in Bryozoa on *Laminaria saccharina* (old blade).

Levring (1937) : Endozoic in *Membranipora* on *Laminaria.*

48. **Entonema aequale** (OLTM.) KYLIN

Rosenvinge & Lund (1941, as *Streblonema aequale* OLTM.), Sundene (1953), J. Feldmann (1954) : On *Chorda filum.*

49. **E. maculans** (P. DANG.) HAMEL

J. Feldmann (1954): On *Laminaria digitata* (endophytic in blade).

50. **Laminariocolax tomentosoides** (FARL.) KYLIN

Jónsson (1901-3, as *Ectocarpus tomentosoides* FARL.): On *Laminaria digitata, L. hyperborea, Alaria esculenta* (stipe).

Printz (1926, as *Ectocarpus tomentosoides* subsp. *norvegicus* GRAN.): On *Laminaria hyperborea* (blade), *L. saccharina.*

Levring (1937), Taylor (1937, 1957) : On *Laminaria.*

Rosenvinge & Lund (1941) : On *Laminaria digitata.*

Sundene (1953) : On *Laminaria saccharina.*

J. Feldmann (1954) : On *Laminaria digitata* (blade).

Jaasund (1957) : On *Laminaria digitata, L. hyperborea, L. saccharina.*

51. **Phaeostroma pustulosum** KUCK.

Jónsson (1901-3) : On *Chorda tomentosa, Laminaria saccharina* and f. *phyllitis.*

Levring (1937) : On *Laminaria saccharina.*

52. **Phaeostroma** sp.

Printz (1926) : On *Laminaria* (old blade).

53. **Pylaiella gardneri** COLLINS

Setchell & Gardner (1925), G. M. Smith (1944) : On *Postelsia palmaeformis.*

54. **P. littoralis** (L.) KJELLM.

Jónsson (1901-3) : On *Alaria esculenta, Saccorhiza dermatodea.*

55. **P. tenella** SETCH. et GARDN.

Setchell & Gardner (1925) : On *Pleurophycus gardneri.*

Scagel (1957) : On *Pleurophycus.*

56. **Streblonema aecidioides** (ROSENV.) FOSL.

Jónsson (1901-3) : On *Laminaria digitata* f. *cucullata, L. saccharina* and f. *phyllitis.*

Taylor (1937, 1957) : On *Laminaria* (endophytic).

57. **S. deformans** (P. DANG.) HAMEL

J. Feldmann (1954) : On *Laminaria digitata* (endophytic in stipe, sometimes causing spiral twist of the stipe).

58. **S. evagatum** SETCH. et GARDN.

Setchell & Gardner (1925), G. M. Smith (1944) : On *Laminaria setchellii.*

Yamada & Tanaka (1944) : On *Laminaria* sp. (among paraphyses).

59. **S. irregulare** SAUND.

Setchell & Gardner (1925) : On *Nereocystis luetkeana.*

60. **S. myrionematoides** SETCH. et GARDN.

Setchell & Gardner (1925) : On *Laminaria setchellii.*

61. **S. pacificum** SAUND.

Setchell & Gardner (1925) : On *Alaria.*

62. **S. rugosum** SETCH. et GARDN.

Setchell & Gardner (1925) : On *Alaria tenuifolia.*

Scagel (1957) : On *Alaria tenuifolia* f. *tenuifolia.*

63. **S. scabiosum** SETCH. et GARDN.

Setchell & Gardner (1925) : On *Nereocystis luetkeana.*

64. **S. stilophorae** CR. var. **caespitosa** ROSENV.

Jónsson (1901-3) : On *Laminaria digitata.*

RALFSIACEAE

65. **Ralfsia deusta** (AG.) J. AG.

Kjellman (1883) : On *Laminaria* (stipe).

66. **R. pusilla** (STRÖMF.) FOSL.

Strömfelt (1888), Norum (1913) (cf. Levring, 1937) : On *Laminaria saccharina.*

Printz (1926) : On *L. saccharina* (blade).

SPHACELARIALES

SPHACELARIACEAE

67. **Sphacelaria caespitula** LYNGB.

Norum (1913) (cf. Levring 1937) : On *Laminaria.*

Rosenvinge & Lund (1950) : On *Laminaria hyperborea* (stipe).

Sundene (1953) : On *Laminaria digitata, L. hyperborea* (stipe).

68. **S. cirrosa** AG.

Sundene (1953) : On *Chorda filum, Laminaria saccharina* (blade).

Printz (1926) : On *Laminaria hyperborea* (stipe).

Levring (1937), Rosenvinge & Lund (1950) : On *Laminaria.*

69. **S. furcigera** KÜTZ.

Rosenvinge & Lund (1950) : On *Laminaria digitata, L. hyperborea.*

70. **S. olivacea** PRINGSH.

Jónsson (1901-3), Kylin (1907, as *Sphacelaria caespitula,* cf. Rosenvinge & Lund, 1950) : On *Laminaria hyperborea* (stipe).

71. **Chaetopteris plumosa** (LYNGB.) KÜTZ.

Kylin (1910) : On *Laminaria hyperborea.*

Printz (1926) : On *Laminaria* (hapteres).

Rosenvinge & Lund (1950) : On *Laminaria* (hapteres), *L. digitata* (stipe).

Svendsen (1959) : On *Alaria.*

72. **Halopteris filicina** (GRAT.) KÜTZ.

J. Feldmann (1954) : On *Laminaria hyperborea* (base of stipe).

CHORDARIALES

ACROTHRICACEAE

73. **Acrothrix gracilis** KYLIN

Rosenvinge & Lund (1943) : On *Laminaria.*

74. **A. pacifica** OKAMURA et YAMADA f. **pacifica** INAGAKI

Yamada (1932), Tokida (1954), Inagaki (1954, 1958) : On *Chorda filum.*

f. **crassa** INAGAKI

Inagaki (1954, 1958) : On *Chorda filum.*

CHORDARIACEAE

75. **Chordaria flagelliformis** (MÜLL.) AG.

Svendsen (1959) : On *Laminaria digitata* (blade).

76. **Papenfussiella kuromo** (YENDO) INAGAKI f. **kuromo** INAGAKI

Inagaki (1958) : On *Eisenia bicyclis.*

CORYNOPHLAEACEAE

77. **Microcoryne ocellatus** STRÖMF.
Rosenvinge & Lund (1943): On *Chorda filum.*

ELACHISTACEAE

78. **Elachista fucicola** (VELL.) ARESCH.
Rosenvinge (1935): On *Laminaria digitata.*

79. **Leptonema fasciculatum** REINKE
Tobler (1909) (cf. Printz, 1926): On *Laminaria.*
Jónsson (1901-3): On *Laminaria, Alaria esculenta, Saccorhiza dermatodea.*
Rosenvinge (1935): *Laminaria digitata.*

MYRIONEMATACEAE

80. **Ascocyclus foecundes** (STRÖMF.) COTTON
Levring (1937): On *Laminaria, Alaria esculenta.*

81. **A. hispanicus** SAUV.
J. Feldmann (1954): On *Laminaria hyperborea* (blade).

82. **A. islandicus** H. JÓNSSON
Jónsson (1901-3): On *Laminaria saccharina.*

83. **A. orbicularis** (J. AG.) MAGNUS
Printz (1926): On *Laminaria digitata.*

84. **A. saccharinae** COTTON
Jaasund (1957): On *Laminaria saccharina.*

85. **Chilionema börgeseni** PRINTZ
Printz (1926): On *Laminaria digitata* (blade).

86. **Clathrodiscus mandoulii** (SAUV.) HAMEL
J. Feldmann (1954): On *Saccorhiza polyschides* (apex of young blade).

87. **Compsonema fructuosum** SETCH. et GARDN.
Setchell & Gardner (1925): On *Nereocystis luetkeana* (cyst).

88. **C. myrionematoides** SETCH. et GARDN.
Setchell & Gardner (1925), G. M. Smith (1944): On *Nereocystis luetkeana* (stipe).

89. **C. nummuloides** SETCH. et GARDN.
Setchell & Gardner (1925): On *Nereocystis luetkeana* (cyst).

90. **C. pusillum** SETCH. et GARDN.
Setchell & Gardner (1925), G. M. Smith (1944): On *Nereocystis luetkeana.*

91. **C. ramulosum** SETCH. et GARDN.
Setchell & Gardner (1925): On *Nereocystis luetkeana.*

92. **C. secundum** SETCH. et GARDN.
Setchell & Gardner (1925), G. M. Smith (1944): On *Nereocystis luetkeana.*
f. **terminale** SETCH. et GARDN.

Setchell & Gardner (1925) : On *Nereocystis luetkeana.*
Yamada & Tanaka (1944) : On *Laminaria* sp., *Alaria angusta.*

93. **C. serpens** SETCH. et GARDN.
G. M. Smith (1944) : On *Egregia menziesii.*

94. **C. sessile** SETCH. et GARDN.
Setchell & Gardner (1925), Scagel (1957) : On *Hedophyllum sessile.*

95. **C. speciosum** (BÖRG.) SETCH. et GARDN. f. **piliferum** SETCH. et GARDN.
Setchell & Gardner (1925) : On *Nereocystis luetkeana.*

96. **C. sporangiiferum** SETCH. et GARDN.
Setchell & Gardner (1925) : On *Nereocystis luetkeana.*

97. **C. streblonematoides** SETCH. et GARDN.
Setchell & Gardner (1925) : On *Nereocystis luetkeana.*
Scagel (1957) : On *Nereocystis* (cyst).

98. **Hecatonema clavatum** SETCH. et GARDN.
Setchell & Gardner (1925) : On *Nereocystis luetkeana.*

99. **H. diffusum** KYLIN
Kylin (1910) : On *Laminaria digitata, L. hyperborea.*
Sundene (1953) : On *Laminaria digitata.*

100. **H. lawsonii** SETCH. et GARDN.
Setchell & Gardner (1925) : On *Nereocystis* (sporophyll).

101. **H. maculans** (COLL.) SAUV.
Printz (1926) : On *Laminaria hyperborea, L. saccharina.*

102. **H. reptans** (CROU.) SAUV.
Levring (1937) : On *Laminaria digitata.*

103. **H. speciosum** (BÖRG.) COTTON
Levring (1937) : On *Laminaria digitata, L. hyperborea.*

104. **H. terminale** (KÜTZ.) KYLIN
Levring (1937): On *Laminaria.*
Sundene (1953) : On *Laminaria digitata.*

105. **H. variabile** SETCH. et GARDN.
Setchell & Gardner (1925), G. M. Smith (1944) : On *Nereocystis luetkeana* (cyst).

106. **Myrionema aecidioides** (ROSENV.) SAUV.
Kylin (1910) : On *Laminaria digitata, L. saccharina.*
Levring (1937) : On *Laminaria saccharina.*

107. **M. alariae** (NORUM) PRINTZ
Norum (1913, as *Phycocelis alariae* NORUM) : On *Alaria.**

*Though Norum's paper has not been consulted by the writer, "*Alaria*" is here listed as the host because the species name of the epiphyte suggests so.

Printz (1926) : On *Laminaria digitata* (endophytic in stipe).

108. **M. attenuatum** SETCH. et GARDN.

Setchell & Gardner (1925), G. M. Smith (1944) : On *Nereocystis luetkeana* (stipe), *Laminaria farlowii.*

f. **doliiforme** SETCH. et GARDN.

Setchell & Gardner (1925) : On *Macrocystis pyrifera* (stipe).

109. **M. balticum** (REINKE) FOSL.

Taylor (1937, 1957) : On *Laminaria.*

110. **M. compsonematoides** SETCH. et GARDN.

Setchell & Gardner (1925), Scagel (1957) : On *Laminaria complanata.*

111. **M. corunnae** SAUV.

Jónsson (1901-3) : On *Laminaria hyperborea* (sorus).
Kylin (1910) : On *Laminaria digitata.*
Setchell & Gardner (1925) : On *Laminaria pallida.*
Taylor (1937, 1957) : On *Laminaria.*
Levring (1937) : On *Laminaria, Alaria esculenta.*
Sundene (1953) : On *Laminaria digitata, L. saccharina.*
Kurogi (1954) : On *Alaria crassifolia.*
J. Feldmann (1954) : On *Laminaria ochroleuca* (blade).
Jaasund (1957) : On *Laminaria digitata* f. *stenophylla.*

f. **angulatum** SETCH. et GARDN.

Setchell & Gardner (1925) : On *Egregia menziesii.*

var. **filamentosa** JÓNSSON

Jónsson (1901-3) : On *Laminaria hyperborea* (sorus).

f. **sterile** SETCH. et GARDN.

Setchell & Gardner (1925), G. M. Smith (1944) : On *Nereocystis luetkeana.*

f. **uniforme** SETCH. et GARDN.

Setchell & Gardner (1925). On *Laminaria sinclairii, Alaria marginata, Costaria costata.*

112. **M. foecundum** (STRÖMF.) FOSL.

Kylin (1910) : On *Laminaria digitata, Alaria esculenta.*
Printz (1926), Taylor (1937, 1957) : On *Laminaria* spp.

f. **divergens** SETCH. et GARDN.

Setchell & Gardner (1925) : On *Nereocystis luetkeana* (sporophyll).

f. **majus** SETCH. et GARDN.

Setchell & Gardner (1925) : On *Laminaria ephemera.*

f. **ramulosum** SETCH. et GARDN.

Setchell & Gardner (1925), Scagel (1957) : On *Nereocystis luetkeana.*

f. **simplissimum** SETCH. et GARDN.
Setchell & Gardner (1925), Scagel (1957) : On *Nereocystis luetkeana* (sporophyll).
f. **subulatum** SETCH. et GARDN.
Setchell & Gardner (1925) : On *Nereocystis luetkeana.*

113. M. **globosum** (REINKE) FOSL.
Printz (1926) : On *Laminaria.*

114. M. **hecatonematoides** SETCH. et GARDN.
Setchell & Gardner (1925) : On *Nereocystis luetkeana* (cyst).

115. M. **laminariae** (ROSENV.) JÓNSSON
Jónsson (1901–3) : On *Laminaria saccharina* f. *phyllitis* (in outer membrane).

116. M. **minutissimum** SETCH. et GARDN.
Setchell & Gardner (1925) : On *Egregia menziesii.*

117. M. **obscurum** SETCH. et GARDN.
Tokida & Yama (1960) : On *Laminaria religiosa* (blade).
Setchell & Gardner (1925) : On *Costaria costata.*

118. M. **papillosum** SAUV.
J. Feldmann (1954) : On *Laminaria digitata* (blade).

119. M. **primarium** SETCH. et GARDN.
Setchell & Gardner (1925) : On *Costaria costata.*
G. M. Smith (1944) : On Laminariales.
f. **acuminatum** SETCH. et GARDN.
Setchell & Gardner (1925) : On *Macrocystis pyrifera.*

120. **Symphiocarpus strangulans** ROSENV.
Kylin (1910) : On *Laminaria hyperborea* (stipe).

DICTYOSIPHONALES

COILODESMACEAE

121. **Coilodesme fucicola** (YENDO) NAGAI
Nagai (1940) : On *Laminaria longipes* (blade).

DICTYOSIPHONACEAE

122. **Dictyosiphon ekmani** ARESCH.
Kylin (1910) : On *Laminaria digitata* (blade).

123. D. **foeniculaceus** (HUDS.) GREV.
Rosenvinge & Lund (1947) : On *Chorda filum.*

GIRAUDIACEAE

124. **Giraudia sphacelarioides** DERB. et SOL.
Rosenvinge & Lund (1947) : On *Chorda filum, Laminaria* (hapteres).

MYRIOTRICHIACEAE

125. **Myriotrichia clavaeformis** HARV.

Rosenvinge & Lund (1947) : On *Chorda filum.*

PUNCTARIACEAE

126. **Asperococcus bullosus** LAMOUR.

Tobler (1909) (cf. Printz, 1926) : On *Laminaria.*

127. **A. echinatus** (MERT.) GREV.

Tobler (1909) (cf. Printz, 1926) : On *Laminaria.*

Rosenvinge & Lund (1947) : On *Laminaria digitata, L. hyperborea.*

f. **vermicularis** (GRIFF.) HARV.

Kylin (1910) : On *Laminaria saccharina* (blade).

128. **A. fistulosus** (HUDS.) HOOK.

Levring (1937) : On *Laminaria.*

129. **Desmotrichum undulatum** (J. AG.) REINKE

Rosenvinge & Lund (1947) : On *Chorda filum, Laminaria saccharina* (young frond).

130. **Halorhipis winstonii** (AND.) SAUND.

G. M. Smith (1944) : On *Egregia laevigata.*

131. **Litosiphon filiformis** (REINKE) BATT.

Printz (1926) : On *Laminaria digitata* f. *stenophylla, L. saccharina.*

Levring (1937), Rosenvinge & Lund (1947), Jaasund (1957) : On *Laminaria saccharina.*

Sundene (1953) : On *Laminaria digitata, L. saccharina.*

132. **L. laminariae** (LYNGB.) HARV.

Kylin (1910), Printz (1926), Levring (1937), J. Feldmann (1954), Jaasund (1957) : On *Alaria esculenta.*

133. **L. pusillus** (CARM.) HARV.

Kylin (1910), Levring (1937), Sundene (1953), J. Feldmann (1954), Jaasund (1957) : On *Chorda filum.*

Printz (1926) : On *Chorda filum, Laminaria saccharina, Saccorhiza bullosa.*

Rosenvinge & Lund (1947) : On *Chorda filum, Laminaria saccharina.*

134. **L. tenuis** LEVRING

Levring (1937), Jaasund (1957) : On *Laminaria digitata.*

135. **L. yezoense** YAMADA et NAKAMURA

Yamada (1944), Yamada & Tanaka (1944) : On *Laminaria* (blade).

136. **Rhadinocladia farlowii** SCHUH

Taylor (1937, 1957) : On *Chorda.*

STRIARIACEAE

137. **Isthmoplea sphaerophora** (CARM.) KJELLM.

Jónsson (1901-3) : On *Laminaria digitata.*

Printz (1926) : On *Laminaria hyperborea* (stipe).

DESMARESTIALES

DESMARESTIACEAE

138. **Desmarestia viridis** (MÜLL.) LAMOUR.

Jónsson (1901-3) : On *Laminaria*.

Printz (1926) : On *Laminaria digitata*.

TILOPTERIDALES

TILOPTERIDACEAE

139. **Tilopteris mertensii** (SMITH) KÜTZ.

Printz (1926) : On *Laminaria* (hapteres).

LAMINARIALES

LAMINARIACEAE

140. **Laminaria angustata** KJELLM. (young plants).

Hasegawa & Fukuhara (1956) : On *Laminaria angustata* (old blade).

141. **L. japonica** ARESCH. (young plants).

Tokida & Ohiwa (1957) : On *Alaria crassifolia* (old stipe).

RHODOPHYCEAE

PROTOFLORIDEAE

PORPHYRIDIALES

PORPHYRIDIACEAE

142. **Goniotrichum elegans** (CHAUV.) LE JOL.

Reinsch (1875, as *Callonema olivaceum* REINSCH, *cf.* Hamel, 1924) : On *Chorda filum*.

Tokida (1954) : On *Chorda*.

BANGIALES

ERYTHROPELTIDACEAE

143. **Erythrocladia subintegra** ROSENV.

Printz (1926) : On *Laminaria digitata* (blade).

144. **Erythrotrichia carnea** (DILLW.) J. AG.

Sundene (1953) : On *Laminaria saccharina*.

145. **E. welwitschii** (RUPR.) BATT.

G. M. Smith (1944) : On *Nereocystis luetkeana* (stipe).

146. **Porphyropsis coccinea** (J. AG.) ROSENV.

Rosenvinge (1909) : On *Laminaria digitata* (stipe).

Printz (1926): On *Laminaria hyperborea* (stipe and blade margin).

BANGIACEAE

147. **Porphyra drachii** J. FELDM.

J. Feldmann (1954): On Laminariales (blade), *Saccorhiza polyschides*, *Laminaria hyperborea*, *L. digitata*.

148. **P. miniata** (LYNGB.) AG.
Printz (1926): On *Laminaria, Alaria pylaii* (stipe).
Levring (1937): On *Laminaria* (stipe).
Taylor (1937, 1957): On *Laminaria* (base).
Svendsen (1959): On *Alaria, Laminaria digitata.*

149. **P. nereocystis** ANDERSON
G. M. Smith (1944), Scagel (1957): On *Nereocystis luetkeana* (stipe).

150. **P. occidentalis** SETCH. et GARDN.
G. M. Smith (1944): On *Pterygophora californica* (stipe).

151. **P. woolhousiae** HARV.
Levring (1953): On *Macrocystis pyrifera.*

152. **Porphyrella gardneri** G. M. SMITH et HOLLENB.
G. M. Smith (1944), Scagel (1957): On *Laminaria setchellii, Egregia menziesii.*

FLORIDEAE

NEMALIONALES

CHANTRANSIACEAE

153. **Acrochaetium daviesii** (DILLW.) NÄG.
Sundene (1953): On *Laminaria digitata.*
Nakamura (1944, as *Rhodochorton daviesii* (DILLW.) DREW): *On Ecklonia.*
J. Feldmann (1954): On *Laminaria hyperborea, Saccorhiza polyschides.*

154. **A. infestans** HOWE et HOYT
J. Feldmann (1954): Endozoic in stolons and hydrocaules of *Obelia geniculata* attached to *Laminaria* frond.

155. **A. laminariae** J. FELDM.
J. Feldmann (1954): On *Laminaria digitata* (stipe, semi-endophytic).

156. **A. pacificum** KYLIN
Scagel (1957): On *Agarum fimbriatum.*

157. **A. plumosum** (DREW) G. M. SMITH
G. M. Smith (1944): On *Laminaria setchellii, Egregia menziesii, Pterygophora californica.*

158. **A. sancti-thomae** BÖRG.
Nakamura (1941, as *Rhodochorton sancti-thomae* (BÖRG.) NAKAMURA): On *Ecklonia cava.*

159. **A. secundatum** (LYNGB.) NÄG.
Sundene (1953): On *Laminaria digitata.*

160. **A. strictum** (ROSENV.) HAMEL
Sundene (1953): On *Laminaria hyperborea* (stipe).

161. **A. variabile** (DREW) G. M. SMITH

G. M. Smith (1944) : On *Laminaria setchellii, Pterygophora californica.*

Scagel (1957) : On *Laminaria* and *Pterygophora,*

162. A. virgatulum (HARV.) J. AG.

J. Feldmann (1954) : On *Laminaria hyberporea.*

163. Audouinella efflorescens (J. AG.) PAPENF.

Sundene (1953, as *Grania efflorescens* (J. AG.) KYLIN) : On *Laminaria saccharina.*

164. Chantransia thuretii (BORN.) KYLIN *β*. **agama** ROSENV.

Rosenvinge (1909) : On *Laminaria* (stipe).

165. Kylinia alariae (Jónsson) Kylin

Jónsson (1901-3, as *Chantransia alariae* JÓNSS.), Kylin (1910), Levring (1937) : On *Alaria esculenta* (blade).

Taylor (1937, 1957, as *Acrochaetium alariae* (JÓNSS.) BORN.) : On *Alaria.*

166. Rhodochorton membranaceum MAGNUS

Jónsson (1901-3) : On *Laminaria.*

167. Rh. penicilliform (KJELLM.) ROSENV.

Jónsson (1901-3) : On *Laminaria.*

168. Rh. purpureum (LIGHTF.) ROSENV.

Taylor (1937, 1957) : On *Laminaria* (base).

169. Rh. repens JÓNSSON

Jónsson (1901-3) : On *Alaria esculenta* (stipe).

170. Rh. rothii (THUR.) NÄG.

Kylin (1910) : On *Laminaria hyperborea* (stipe).

Rosenvinge (1923-4) : On *Laminaria hyperborea, L. digitata* (stipe and hapteres).

Hamel (1928) : On *Laminaria saccharina* (stipe).

Levring (1937) : On *Laminaria* (stipe).

171. Rh. sparsum (CARM.) KJELLM.

Kjellman (1883) : On Laminariaceae (stipe).

Tobler (1909) (cf. Printz, 1926) : On *Laminaria.*

172. Rh. velutinum (HAUCK) HAMEL

Hamel (1928) : On *Laminaria hyperborea.*

CRYPTONEMIALES

DUMONTIACEAE

173. Dumontia incrassata (MÜLL.) LAMOUR.

Printz (1926) : On *Laminaria hyperborea* (old blade).

SQUAMARIACEAE

174. Cruoriella armorica CROUAN

J. Feldmann (1954) : On *Laminaria hyperborea* (stipe).

175. Peyssonelia rosenvingii SCHMITZ

Jónsson (1901-3) : On *Laminaria hyperborea* (hapteres).

176. **Rhododermis elegans** CROUAN

Rosenvinge (1917) : On *Laminaria digitata* (hapteres).

G. M. Smith (1944) : On *Pterygophora californica* (sporophyll).

177. **Rh. parasitica** BATT.

Jónsson (1901-3) : On *Laminaria hyperborea* (stipe).

Taylor (1937, 1957) : On *Laminaria*.

Nagai (1941) : On *Laminaria platymeris, Arthrothamnus kurilensis, Alaria dolichorhachis, Thalassiophyllum clathrus* (holdfast, stipe).

CRUORIACEAE

178. **Cruoria pellita** (LYNGB.) FRIES

Printz (1926) : On *Laminaria* (hapteres, stipe).

Levring (1937) : On *Laminaria* (stipe).

179. **Petrocelis hennedyi** (HARV.) BATT.

Jónsson (1901-3), Kylin (1910), Rosenvinge (1917), Levring (1937) : On *Laminaria hyperborea* (stipe).

Printz (1926) : On *Laminaria hyperborea, L. digitata* (stipe).

KALLYMENIACEAE

180. **Callophyllis laciniata** (HUDS.) KÜTZ.

Levring (1937) : On *Laminaria* (stipe).

Sundene (1953) : On *Laminaria hyperborea.*

J. Feldmann (1954) : On *Laminaria hyperborea* (stipe).

181. **Cirrulicarpus gmelini** (GRUN.) TOKIDA et MASAKI

Nagai (1941, as *Erythrophyllum gmelini* (GRUN.) YENDO) : On *Laminaria ochotensis, Alaria fistulosa, Thalassiophyllum clathrus* (holdfast).

182. **Euthora cristata** (L.) J. AG.

Kjellman (1883): On *Laminaria digitata.*

Jónsson (1901-3) : On *Laminaria* (hapteres), *L. hyperborea.*

Kylin (1910) : On *Laminaria hyperborea* (stipe).

Rosenvinge (1931) : On *Laminaria hyperborea, L. saccharina* (hapteres).

Levring (1937), Sundene (1953) : On *Laminaria hyperborea.*

Taylor (1957) : On *Laminaria* (base).

183. **E. fruticulosa** (RUPR.) J. AG.

Kawabata (1931) : On *Agarum cribrosum.*

184. **Kallymenia oblongifructa** SETCH.

Scagel (1957) : On *Pleurophycus, Pterygophora* (stipe).

CORALLINACEAE

185. **Clathromorphum circumscriptum** (STRÖMF.) FOSL.

Jónsson (1901-3) : On *Laminaria* (hapteres).

186. **Corallina officinalis** L.

Kjellman (1883) : On *Laminaria*.

187. **Dermatolithon ascripticium** (FOSL.) SETCH. et L. R. MASON

Mason (1953) : On *Laminaria sinclairii* (stipe).

188. **D. canescens** (FOSL.) FOSL.

Masaki & Tokida (1960) : On *Alaria crassifolia* (hapteres).

189. **D. crouanii** (FOSL.) HAMEL et LEMOINE

Jónsson (1901-3, as *Lithophyllum crouanii* FOSL.) : On *Laminaria hyperborea* (stipe).

Foslie (1905) : On *Laminaria digitata, L. hyperborea* (hapteres, stipe).

190. **D. macrocarpum** (ROSAN.) FOSL.

Kjellman (1883, as *Melobesia macrocarpa* ROSAN.) : On *Laminaria digitata*.

Jónsson (1901-3) : On *Laminaria hyperborea* (stipe).

f. **laminariae** (CR.) FOSL.

Foslie (1898, as *Melobesia laminariae* CR.): On *Laminaria hyperborea* (stipe).

191. **D. pustulatum** (LAMOUR.) FOSL.

Suneson (1943) : On *Laminaria hyperborea* (hapteres).

f. **laminariae** (CR.) FOSL.

J. Feldmann (1954) : On *Laminaria hyperborea* and *L. digitata* (hapteres, base of stipe).

192. **Epilithon membranaceum** (ESP.) HEYDR.

Foslie (1905, as *Lithothamnium membranaceum* (ESP.) FOSL.): On *Laminaria digitata* (stipe).

Suneson (1943) : On *Laminaria digitata*.

Sundene (1953) : On *Laminaria saccharina*.

193. **Heteroderma nicholsii** SETCH. et L. R. MASON.

Mason (1953) : On *Eisenia arborea*.

194. **Lithophyllum orbiculatum** (FOSL.) FOSL.

Suneson (1943) : On *Laminaria hyperborea* (hapteres).

195. **Lithothamnium eckloniae** FOSL.

Foslie (1902, as *Lithothamnium capense* f. *eckloniae* FOSL.) : On *Ecklonia* (stipe).

196. **Lithoth. haptericolum** FOSL.

Foslie (1906) : On *Ecklonia* (hapteres).

197. **Lithoth. japonicum** FOSL.

Foslie (1899), Yendo *in* Okamura (1936): On *Laminaria* (hapteres).

198. **Lithoth. vescum** FOSL.

Foslie (1907): On *Ecklonia* (hapteres).
Yendo *in* Okamura (1936): On *Ecklonia cava* (hapteres).

199. **Lithothamnium** sp.
Svendsen (1959): On *Laminaria* (hapteres).

200. **Melobesia farinosa** LAMOUR. (See "Addendum" on p, 105).
Rosanoff (1866): On *Ecklonia radiata.*
Masaki & Tokida (1960): On *Undaria undarioides* (hapteres).

201. **M. limitata** (FOSL.) ROSENV.
Rosenvinge (1917): On *Laminaria digitata.*
Levring (1937), Sundene (1953): On *Laminaria.*
Suneson (1943): On *Laminaria saccharina, L. hyperborea* (blade).

202. **M. minutula** FOSL. f. **lacunosa** FOSL.
Suneson (1943): On *Laminaria saccharina.*

203. **Phymatolithon polymorphum** (L.) FOSL.
Printz (1926): On *Laminaria* (hapteres, stipe).

GIGARTINALES

GIGARTINACEAE

204. **Chondrus crispus** (L.) STACKH.
Rosenvinge (1931): On *Laminaria hyperborea* (stipe).

PHYLLOPHORACEAE

205. **Phyllophora epiphylla** (FL. DAN.) BATT.
Levring (1937): On *Laminaria* (stipe).

206. **Ph. palmettoides** J. AG.
J. Feldmann (1954): On *Laminaria hyperborea* (old stipe).

PLOCAMIACEAE

207. **Plocamium coccineum** (HUDS.) LYNGB.
Kylin (1910), J. Feldmann (1954): On *Laminaria hyperborea* (stipe).
Rosenvinge (1931): On *Laminaria hyperborea.*
Sundene (1953): On *Laminaria hyperborea* (hapteres).
Printz (1926): On *Laminaria* (hapteres, stipe).
Levring (1937): On *Laminaria* (stipe).

RHODOPHYLLIDACEAE

208. **Cystoclonium purpurascens** (HUDS.) KÜTZ.
Printz (1926): On *Laminaria* (stipe).

209. **Rhodophyllis bifida** (GOOD. et WOODW.) KÜTZ.
Levring (1937): On *Laminaria hyperborea.*

210. **Rh. dichotoma** (LEPECH.) GOBI
Kjellman (1883): On Laminariaceae.

BONNEMAISONIALES

BONNEMAISONIACEAE

211. **Bonnemaisonia asparagoides** (WOODW.) AG.

Rosenvinge (1923-4) : On *Laminaria saccharina.*

Printz (1926) : On *Laminaria saccharina* (hapteres, stipe).

212. **B. intricata** (AG.) SILVA (sporophyte)

Printz (1926, as *Trailliella intricata* (AG.) BATT.): On *Laminaria* (hapteres, stipe).

Sundene (1953, as *Trailliella intricata*) : On *Laminaria saccharina.*

RHODYMENIALES

CHAMPIACEAE

213. **Gastroclonium ovatum** (HUDS.) PAPENF.

J. Feldmann (1954) : On *Laminaria* (base).

214. **Lomentaria articulata** (HUDS.) LYNGB.

J. Feldmann (1954) : On *Laminaria hyperborea* (stipe).

215. **L. clavellosa** (TURN.) GAIL

Jónsson (1901-3) : On *Laminaria hyperborea* (hapteres).

Levring (1937) : On *Laminaria hyperborea.*

J. Feldmann (1954) : On *Laminaria hyperborea* (stipe).

216. **L. rosea** (HARV.) THUR.

Jónsson (1901-3) : On *Laminaria hyperborea* (hapteres).

Levring (1937) : On *Laminaria hyperborea.*

RHODYMENIACEAE

217. **Botryocladia pseudodichotoma** (FARL.) KYLIN

G. M. Smith (1944) : On *Macrocystis pyrifera* (holdfast).

218. **Rhodymenia palmata** (L.) GREV.

Rosenvinge (1931), Sundene (1953) : On *Laminaria digitata, L. hyperborea* (stipe).

J. Feldmann (1954) : On *Laminaria hyperborea* (stipe).

var. **mollis** SETCH. et GARDN.

G. M. Smith (1944) : On *Nereocystis luetkeana* (stipe).

f. **prolifera** KÜTZ.

Kylin (1910) : On *Laminaria hyperborea* (stipe).

219. **Rh. pseudopalmata** (LAM.) SILVA

J. Feldmann (1954) : On *Laminaria hyperborea* (stipe).

CERAMIALES

CERAMIACEAE

220. **Antithamnion boreale** (GOBI) KJELLM.

Printz (1926) : On *Laminaria.*

221. **A. defectum** KYLIN

G. M. Smith (1944) : On *Nereocystis luetkeana* (stipe).

222. **A. glanduliferum** KYLIN
G. M. Smith (1944) : On *Nereocystis luetkèana* (stipe).
Scagel (1957) : On *Laminaria* (blade).

223. **A. occidentale** KYLIN
G. M. Smith (1944) : On *Nereocystis luetkeana* (stipe).
Scagel (1957) : On *Nereocystis*.

224. **A. pacificum** (HARV.) KYLIN
G. M. Smith (1944), Scagel (1957) : On *Nereocystis luetkeana* (stipe).

225. **A. plumula** (ELLIS) THUR.
Printz (1926) : On *Laminaria* (hapteres, blade).

226. **A. uncinatum** GARDN.
G. M. Smith (1944) : On *Macrocystis pyrifera, Nereocystis luetkeana*.

227. **Antithamnion** sp.
Nagai (1941) : On *Alaria fistulosa* (holdfast).

228. **Callithamnion corymbosum** (SMITH) LYNGB.
Printz (1926) : On *Laminaria* (blade).
J. Feldmann (1954) : On *Chorda filum*.

229. **C. polyspermum** AG.
Printz (1926): On *Laminaria*.

230. **C. tetragonum** (WITH.) AG. var. **brachiatum** (BONN.) J. AG.
J. Feldmann (1954): On *Laminaria hyperborea* (stipe, blade).

231. **Campylaephora hypnaeoides** J. AG. f. **typica** NAKAMURA
Nakamura (1950) : On *Laminaria*.
f. **hamata** (COTTON) NAKAMURA
Nakamura (1950) : On *Laminaria*.
Tokida & Yama (1960) : On *Laminaria religiosa* (blade).

232. **Ceramium areschougii** KYLIN
Kylin (1910) : On *Laminaria* spp. (blade).

233. **C. deslongchampsii** CHAUV.
Levring (1937) : On *Laminaria digitata*.

234. **C. rubrum** (HUDS.) AG.
Printz (1926) : On *Alaria*.
Sundene (1953) : On *Chorda filum, Laminaria* spp.

235. **C. secundum** LYNGB.
Printz (1926) : On *Laminaria* (stipe).

236. **C. septentrionalis** HENNING
Printz (1926) : On *Laminaria* (blade).

237. **C. strictum** GREV. et HARV.
Tobler (1909) (cf. Printz, 1926) : On *Laminaria.*
Sundene (1953) : On *Chorda filum, Laminaria* spp.

238. **C. tenuissimum** (LYNGB.) J. AG.
J. Feldmann (1954) : On *Chorda filum.*

239. **Microcladia californica** FARLOW
G. M. Smith (1944) : On *Egregia.*

240. **Neoptilota asplenioides** (TURN.) KYLIN
Nagai (1941) : On *Laminaria longipes, Alaria fistulosa, Pleuropterum paradiseum, Thalassiophyllum clathrus* (holdfast).

241. **Pleonosporium kobayashii** OKAMURA
Okamura (1933) : On *Thalassiophyllum clathrus* (holdfast).

242. **Plumaria elegans** (BONN.) SCHMITZ
Taylor (1937, 1957) : On *Laminaria* (base).

243. **Ptilota pectinata** (GUNN.) KJELLM.
Printz (1926) : On *Laminaria* (stipe).
Nagai (1941) : On *Thalassiophyllum clathrus* (holdfast).

244. **P. plumosa** (L.) Ag.
Kylin (1910) : On *Laminaria hyperborea* (stipe).
Printz (1926) : On *Laminaria* (hapteres, stipe).
Levring (1937) : *Laminaria hyperborea.*
Sundene (1953) : On *Laminaria digitata, L. hyperborea* (stipe).

245. **P. serrata** KÜTZ.
Taylor (1937, 1957) : On *Laminaria* (about the base).

246. **Ptilothamnion pluma** (DILLW.) THUR.
J. Feldmann (1954) : On *Laminaria hyperborea* (stipe).

247. **Spermothamnion repens** (DILLW.) ROSENV.
Rosenvinge (1923-4) : On *Laminaria digitata, L. hyperborea.*

DELESSERIACEAE

248. **Acrosorium reptans** (CR.) KYLIN
J. Feldmann (1954): On *Laminaria hyperborea* and *L. digitata* (hapteres, stipe).

249. **Apoglossum ruscifolium** (TURN.) J. AG.
Sundene (1953): On *Laminaria hyperborea.*

250. **Delesseria kurilensis** RUPR. (?)
Ruprecht (1851) : On (?) *Thalassiophyllum.*

251. **D. sanguinea** (L.) LAMOUR.
Kjellman (1883, as *Hydrolapathum sanguineum* (L.) STACKH.): On *Laminaria* spp.

Börgesen (1902) : On *Laminaria hyperborea.*

Printz (1926) : On *Laminaria, Alaria* (stipe).

252. **Hymenena smithii** KYLIN

G. M. Smith (1944) : On *Pterygophora californica* (stipe).

253. **Hypoglossum woodwardii** KÜTZ.

J. Feldmann (1954) : On *Laminaria hyperborea* (stipe).

254. **Membranoptera alata** (HUDS.) STACKH.

Kjellman (1883, as *Delesseria alata* (HUDS.) LAMOUR.): On Laminariaceae.

Börgesen (1902) : On *Laminaria hyperborea.*

Jónsson (1901-3), Kylin (1910), J. Feldmann (1954) : On *Laminaria hyperborea* (stipe).

Printz (1926), Levring (1937) : On *Laminaria* (stipe).

Rosenvinge (1923-4) : On *Laminaria.*

255. **M. denticulata** (MONT.) KYLIN

Nagai (1941) : On *Alaria fistulosa* (stipe).

256. **M. spinulosum** (RUPR.) J. AG.

Kjellman (1883, as *Delesseria montagnei* KJELLM.): On Laminariaceae.

257. **M. weeksiae** SETCH. et GARDN.

G. M. Smith (1944) : On *Pterygophora californica.*

258. **Pantoneura angustissima** (GRIFF.) KYLIN

Taylor (1937, 1957) : On *Laminaria* (base).

259. **P. baerii** (POST. et RUPR.) KYLIN

Kjellman (1883, as *Delesseria baerii* RUPR.) : On Laminariaceae (hapteres).

260. **P. corymbosa** (J. AG.) KYLIN

Kjellman (1883, as *Delesseria corymbosa* J. AG.) : On *Laminaria saccharina* (stipe).

261. **Phycodrys rubens** (HUDS.) BATT.

Rosenvinge (1923-4) : On *Laminaria.*

J. Feldmann (1954) : On *Laminaria hyperborea* (stipe).

262. **Ph. sinuosa** KÜTZ.

Ruprecht (1851, as *Delesseria crenata* RUPR.): On *Laminaria* (hapteres).

Kylin (1910, as *Delesseria sinuosa* (GOOD. et WOODW.) LAMOUR.) : On *Laminaria hyperborea* (stipe).

Printz (1926): On *Laminaria* (hapteres, stipe).

Levring (1937): On *Laminaria.*

263. **Yendonia crassifolia** (RUPR.) KYLIN

Ruprecht (1851, as *Delesseria crassifolia* RUPR.) : Among rhizome of *Laminaria longipes.*

DASYACEAE

264. **Heterosiphonia plumosa** (ELLIS) BATT.
J. Feldmann (1954) : On *Laminaria hyperborea* (stipe).

RHODOMELACEAE

265. **Amplisiphonia pacifica** HOLLENBERG
Hollenberg (1939), Scagel (1953) : On Laminariales (holdfast).
Scagel (1957) : On *Pterygophora*.

266. **Brongniartella byssoides** (GOOD. et WOODW.) SCHMITZ
Sundene (1953) : On *Laminaria* spp.

267. **Chondria dasyphylla** (WOODW.) AG.
Tokida (1954) : On *Laminaria* (stipe).

268. **Laurencia obtusa** LAMOUR.
Tokida & Yama (1960): On *Laminaria religiosa* (blade).

269. **L. pinnatifida** (GMEL.) LAMOUR.
Rosenvinge (1923-4) : On *Chorda filum*, *Laminaria digitata* (blade).

270. **Odonthalia dentata** (L.) LYNGB.
Printz (1926) : On *Laminaria*, *Alaria* (hapteres, stipe).

271. **Placophora binderi** (J. AG.) J. AG.
Baardseth (1941) (cf. Scagel, 1953) : On *Laminaria* (stipe).

272. **Pl. eckloniae** POCOCK
Scagel (1953) : On *Ecklonia maxima* (stipe).

273. **Polysiphonia arctica** J. AG.
Taylor (1937,1957) : On *Laminaria* (about the base).

274. **P. denudata** (DILLW.) KÜTZ.
J. Feldmann (1954) : On *Chorda filum*.

275. **P. elongata** (HUDS.) HARV.
Rosenvinge (1923-4) : On *Laminaria digitata*.
Printz (1926) : On *Laminaria* (stipe, blade).
Levring (1937) : On *Laminaria* (stipe).

276. **P. flexicaulis** (HARV.) COLLINS
Taylor (1937, 1957) : On *Laminaria*.

277. **P. japonica** HARV.
Segi (1951), Tokida (1954) : On *Laminaria*.
Tokida & Yama (1960) : On *Laminaria japonica*, *L. religiosa* (blade).

278. **P. nigrescens** (SMITH) GREV.
Sundene (1953) : On *Laminaria digitata* and other species.

279. **P. parasitica** (HUDS.) GREV.
Jónsson (1901-3) : On *Laminaria hyperborea* (hapteres).

280. P. urceolata (LIGHTF.) GREV.

Jónsson (1901-3), Levring (1937) : On *Laminaria* (stipe).

Kylin (1910), Rosenvinge (1923-4) : On *Laminaria hyperborea* (stipe).

Printz (1926) : On *Laminaria, Alaria* (hapteres, stipe).

Veldkamp (1950) : On *Laminaria.*

var. **patens** (DILLW.) J. AG.

J. Feldmann (1954) : On *Laminaria hyperborea* (stipe).

281. P. violacea (ROTH) GREV.

Rosenvinge (1923-4) : On *Chorda filum.*

Printz (1926) : On *Laminaria* (stipe, blade).

Sundene (1953) : On *Chorda filum, Laminaria* spp.

J. Feldmann (1954) : On *Chorda filum, Saccorhiza polyschides.*

282. Pterosiphonia parasitica (HUDS.) FALK.

Printz (1926) : On *Alaria esculenta* (hapteres).

Levring (1937) : On *Laminaria hyperborea.*

283. Rhodomela lycopodioides (L.) AG.

Levring (1937) : On *Laminaria.*

f. **typica** KJELLM. subf. **compacta** KJELLM.

Kylin (1910) : On *Laminaria hyperborea.*

284. Rh. subfusca (WOODW.) AG.

Rosenvinge (1923-4) : On *Laminaria.*

285. Taenioma clevelandii FARLOW

G. M. Smith (1944) : On *Nereocystis luetkeana* (holdfast).

Summary

In the present paper are enumerated 285 species of epiphytic marine algae which have been reported in 69 publications to grow upon or in the thallus of Laminariales plants belonging to 22 genera. Of these epiphytes, 2 genus and 3 species belong to Cyanophyceae, 16 genera and 22 species to Chlorophyceae, 37 genera and 116 species to Phaeophyceae, and 71 genera and 144 species to Rhodophyceae. Some relations between the hosts and the epiphytes are considered.

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Addendum : After the manuscript was sent to press, Ass. Professor T. Masaki collected dy dredging at depths of about 30 m. a number of specimens of *Laminaria japonica* in the western part of Aomori Bay, off Imabetsu-machi, Aomori prefecture, on November 5, 1960. The specimens were all densely infested on the surface of the blade with reddish purple thin crusts of *Melobesia farinosa* and with greenish-grey colonies of a Bryozoa.

石狩川口附近の汐目に於ける幼稚魚

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Larvae and young fishes collected around the current rip in Ishikari Bay.

Kiyu KOBAYASHI

Abstract

Larvae and young fishs of 13 families and 17 species including one adult species and two unidentified species were collected from around the current rip, that developed from the mouth of the Ishikari river in Ishikari Bay, on August 1st, 1959.

Specimens were collected with a larva net (130cm × 450cm) by surface tow for 30 minutes, at five stations indicated in Fig. 1.

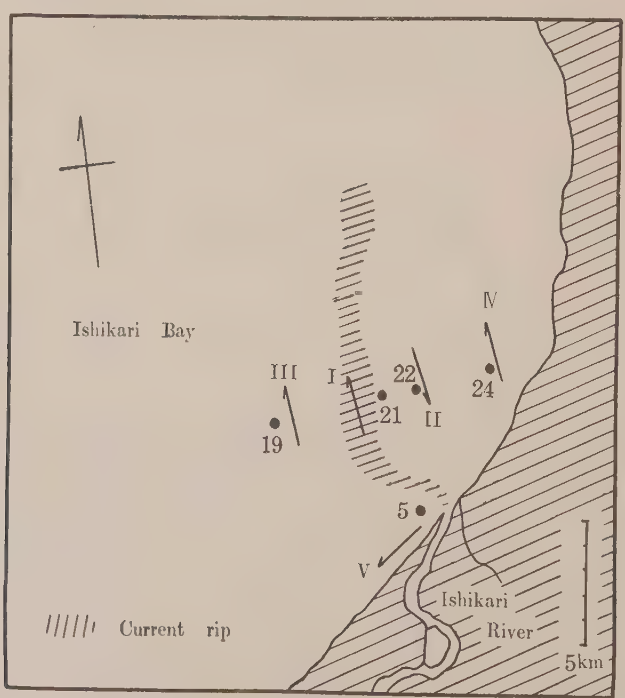


Fig. 1. Stations of fish-larva net tow (Roman numerals) and oceanographical observations (Arabic numerals), on August 1st, 1959

In station I, towed on the current rip, all species that were collected in this research were observed, as presented in table 2.

More species and specimens were collected from the waters to the east side of current rip than to the west side of it. But, this matter does not necessarily indicate fish formations on

either side of the current rip for collected species. (The collection was carried out between 11 a.m. and 3 p. m.)

Many larvae and young of *Engraulis japonica*, *Gasterosteus aculeatus aculeatus*, *Pneumatophorus japonicus japonicus* and 4 species belonging to genus *Sebastes* were collected in this research. It is notable that larvae of *Hippocampus japonicus* and *Stephanolepis cirrhifer* are uncommonspecies in this water.

汐目に種々の漂流物，プランクトン又魚群の集る事は古くより知られ，これに関連した研究が行われているが，最近においては松平・その他（'56 a.b.c [24,25,26]）によつて汐境における研究が行われ報告された。

北海道石狩湾には石狩川の影響によつて川口附近に顕著な汐目が出現するが，未だその構造その他に関する研究調査は無く，1958年以降石田等によつて研究が行われその一部は既に報告された〔石田・その他（'60）[2]鈴木（'60）[39]，鈴木・佐野（'60）[40]〕。

日本海水域における幼稚魚の研究は，近年対馬暖流開発調査の一部として九州大学内田研究室を中心とする研究グループによつて行われその成果が報告されている（対馬暖流開発調査報告書　第2輯，1958，水産庁）。又多くの魚種の卵及び稚魚の形態及び生態が明らかにされた〔内田・その他（'58）[19]〕石狩湾も当然この調査の一部に含まれ，担当研究者によつてこの水域における魚種の卵及び稚魚の分布等が報告されているが，石狩川口附近の湾奥部における資料は少い。

筆者は石田等の研究調査の一部として採集された幼稚魚の資料を見る機会を得たので此処に報告する。採集は1959年8月1日石狩川口より沖合に出現した汐目の上とその両側における水域において稚魚ネット（口径130cm，長さ450cm）を30分間表層曳網した。曳網位置は第1図及び第1表に示す様に汐目上の1点と，海水の影響の強いと見られる西側の2点及び河水の影響の強いと見られる東側の2点の計5点において行われた（採集は午前11時から午後3時の間に行われた）。

Table 1. Data on temperature, salinity and submarine illumination observed at stations on either side of the current rip, that developed from the mouth of the Ishikari river in Ishikari Bay, on August 1st, 1959. The observations were made by Messrs. T. Suzuki and N. Sano 〔Suzuki & Sano ('60)〕.

Items	Station / Depth (m)	* 5	19	21	22	24
W. T. (°C)	0	16.7	18.6	18.1	17.6	16.5
	2.5	16.5	17.5	17.2	17.1	16.4
	5.0		17.2	17.0	16.7	16.2
	10.0		16.7	16.9	15.4	15.6
Salinity (‰)	0	34.239	33.666	28.024	29.785	34.121
	2.5	34.108	33.836	32.978	33.468	33.926
	5.0		33.860	33.235	33.982	33.951
	10.0		33.912	33.764	33.849	34.054
Submarine illumination (LUX)	Surface	95.000	120.000	125.000	115.000	100.000
	0.1	70.000	70.500	28.000	20.000	33.000
	2.5	43.000	70.500	20.500	12.500	18.700
	5.0		58.600	10.400	11.000	17.580
	10.0		26.600	6.600	2.500	7.100

* Depth : 3m

得られた魚種は13科17種及び魚種不明2種で，第2表に見られる様にその総ての種類が汐目上の採集点1において得られており，河水の影響の強いと考えられる採集点2，4において8種が得られ，また海洋の影響の強いと考えられる採集点3，5においては殆んど採集されなかつた。この様な汐目の両側の水域における稚魚の出現状況の明らかな差異の原因は，この場合照度に示される様に，両水域の濁りの差異による様に考えられるが，出現した魚種の組成から見て，日中の深浅移動をも考え併せれば，この様な魚種出現状況の差異が，それぞれの水域の魚種の分布状態を表わすと考える事は早計で，両水域における各層採集また表層の日中採集等をも行う事によつて確められなければならない。

なお本研究に際し，種々御指導を受けた本学部佐藤信一教授に深謝すると共に，本研究の資料の提供を受け種々御便宜を賜つた本学部石田正己助教授，鈴木恒由講師他同研究室の諸氏に感謝する。

Table 2. Table of species by collection stations

Scientific name	Station of fish-larva net tow				
	V	III	I	II	VI
Engraulis japonica	○		○	○	○
Cololabis saira	○		○		
Hemiramphus sajori			○	○	○
Gasterosteus aculeatus aculeatus			○	○	
Syngnathus schlegeli			○	○	○
Hippocampus japonicus			○		
Mugilidae *gen. sp.*			○		○
Pneumatophorus japonicus japonicus			○	○	
Girella punctata			○		
Rhinogobius gymnauchen			○		
Stephanolepis cirrhifer			○		
Fugu sp.			○		
Sebastes taczanowskii			○		
S. pachycephalus			○		
S. sp. A.			○	○	
S. sp. B.			○		
Paralichthys olivaceus			○		○
Unidentified A.			○		
Unidentified B.			○		
Egg of *Cololabis saira*			○	○	

Engraulidae　カタクチイワシ科

Engraulis japonica (Houttuyn)　カタクチイワシ

本種の幼稚魚は1735個体が得られ，その全長範囲は3.5～21.5mmにわたり，その組成は第2図に示す様に汐目上の採集点1及び汐目の両側の採集点5において得られたものは個体数も少く，また全長も汐目の東側の採集点2，4におけるものよりも小さい。

本水域において5～10月に本種の濃厚な産卵の行われる事は対馬暖流開発調査によつて明らかにされている〔Shimomura & Fukataki ('57)[38]〕。なお本種の卵及び稚魚の形態については内田・その他 ('58)[19]による報告がある。また南樺太・南千島水域からの資料に基いて Казанова ('59)[20]の報告がある。

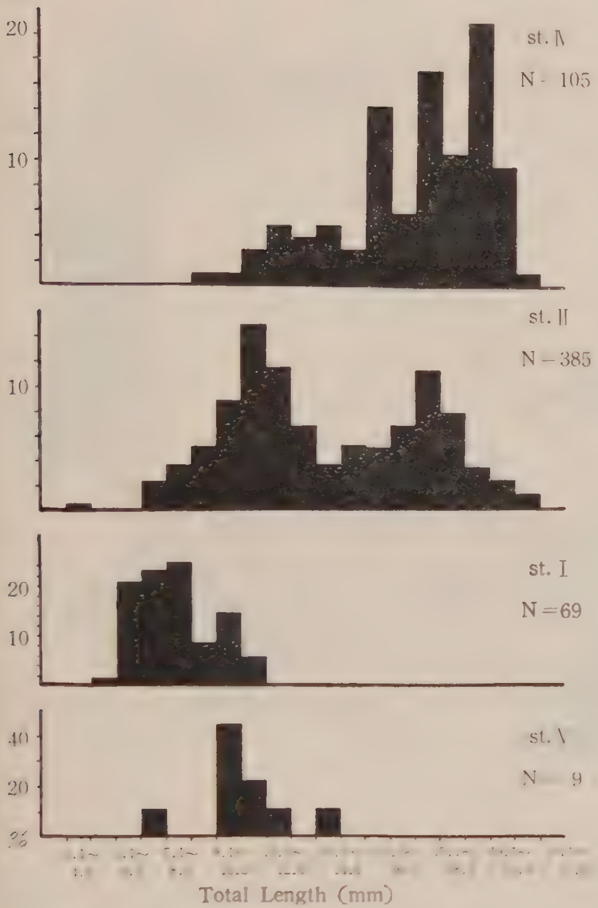


Fig. 2. Frequency distribution of total length of *Engraulis japonica* (Houttuyn) by collection stations

Scombresocidae　サンマ科

Cololabis saira (Brevoort) サンマ

本種の稚魚は汐目上の採集点1において，全長約5.0mmの孵化直後のものと思われるものが9個体得られ，また採集点1及び2において卵が採集された。なお本種の大きな産卵場が北海道西海域に5～7月に形成される事は対馬暖流開発調査によつて明らかにされ，深滝（'58）[8]による報告がある。また卵及び稚魚の形態については内田・その他（'58）[49]，遊佐（'58）[52]及びKазанова（'59）[20]による報告がある。

Hemiramphidae　サヨリ科

Hemiramphus sajori (Temminck & Schlegel) サヨリ

本種の幼稚魚は全長5.0～70.0mm（下顎を含む）の28個体が得られ，孵化直後のものと思われるものから下顎の突出の完成した各発育段階のものが含まれる。なお本種の卵及び稚魚の形態については内田・その他（'58）[48]及び遊佐（'58）[52]等の他数多くの報告がある。

Gasterosteidae　トゲウオ科

Gasterosteus aculeatus aculeatus (Linné) イトヨ

本種の幼稚魚は全長14.8～30.0mmにわたる902個体が得られ，今回の採集に[illegible]最も個体数の多く出現した種類でしかもその殆んど大部分の個体が汐目上の採集点1において得られた。これ等の資料の各採集点における全長組成は第3図に[illegible]約20mmの個体では鱗状板の発達は不完全[illegible]また全長約22mmの個体では鱗状板は完成し，33～34個が明瞭に数えられる[illegible]

なお本種の[illegible]内田・[illegible]（'58）[illegible]19.8mmの標本に関する記載がある。

Syngnathidae　ヨウジウオ科

Syngnathus schlegeli Kaup ヨウジウオ

本種は全長188～195mmの3個体の成魚と[illegible]7.0～38.8mmの9個体[illegible]成魚は1尾の雌と，[illegible]中の1尾の雄並びに育児嚢が空の雄1尾で，稚魚は中村（'35a）[illegible]及び高井・瀧上（'59）[illegible]により放出直後のものと考えられる全長7～11mmの5個体を含む9個体である。なお全長28.0mm及び38.8mmの2個体の幼魚を除く総てが汐目上の採集点1において採集され[illegible]

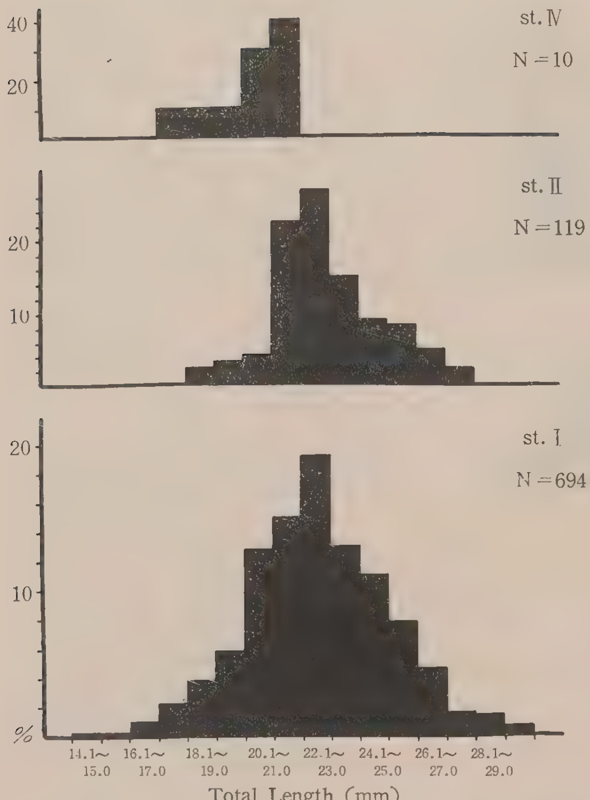


Fig. 3. Frequency distribution of total length of *Gasterosteus aculeatus aculeatus* (LINNÉ) by collection stations



Fig. 4. *Hippocampus japonicus* KAUP
T. L. ca. 12mm

Hippocampus japonicus KAUP キタノウミウマ

本種の幼魚は全長約12mm（頭長3.5mm；軀幹部5.0mm；尾部4.4mm）のものが汐目上の採集点1において1個体得られた。なお標本は中村（'37 b）[35] によつて小湊から報告されたものと大きさその他の特徴は殆んど一致する（Fig. 4）。

Mugilidae ボラ科

Mugilidae *gen. sp.*

本科に属するものは全長9.3〜19.1mmの7個体の標本が得られた。従来 Mugilidae ボラ科に属するものは，北海道沿岸水域には *Mugil cephalus* L. ボラ及び *Liza haematocheila* (T. & S.) メナダの2種の棲息する事が知られているが，これ等ボラ科の幼稚魚については中村（'37 a）[34] によつて小湊附近から *M. cephalus* L. ボラ，*L. carinata* (C. & V.) セスヂボラ，*L. macrolepis* (A. SMITH) コボラ及び *Crenimugil crenilabis* (FORSKÅL) フウライボラ等の稚魚が報告され，また内田・その他（'58）[48] の報告中に *M. cephalus* ボラの全長5.2mm，8.6mm及び19.8mmのものに関する記載がある。その報告中に近縁の *M. japonicus* T. & S. カラスミボラとの異同に疑問を残しているが，石山（'51）[13] により明らかにされている様に，特に本科魚類の同定は外部形態によつてのみ行う事は困難であり，現在の段階では同定は不可能であるので，本報告においては種名不明のまま記録する。

Scombridae　サバ科

Pneumatophorus japonicus japonicus (HOUTTYN)　マサバ

本種の幼稚魚は汐目上の採集点1において100個体及び汐目の東側の採集点2において20個体計120個体が得られ，全長範囲3.0～25.5mmにわたる各発育段階のものが含まれている。

マサバの北海道西海域における卵及び稚魚に関しては，対馬暖流開発調査により調査研究が行われ，石狩湾水域においては6～8月に卵及び稚魚が多量に出現する事が明らかにされ，Motoda & Anraku ('55)[28]，佐野・田村・飯塚 ('56)[37] 及び Shimomura & Fukataki ('57)[8] 等の報告がある。また卵及び稚魚の形態に関しては内田・その他 ('58)[45] の研究報告の中に詳述されている。なお Pacc ('59)[36] 及び Дехник ('59)[2] によって南樺太・南千島水域における卵及び稚魚の生態，またそれ等の資料に基く形態等が明らかにされた。

Girellidae　メジナ科

Girella punctata GRAY　メジナ

本種の稚魚は全長 20.0mm の1個体が汐目上の採集点1において得られた。鰭条は何れも完成し，背鰭 XV,13；臀鰭 III,13；胸鰭 21；腹鰭 I,5 がそれぞれ数えられる (Fig. 5)。

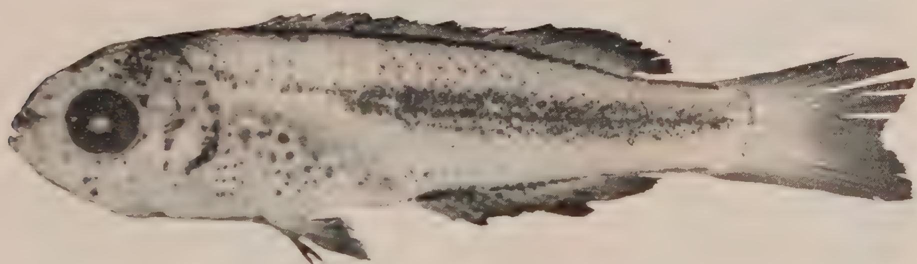


Fig. 5. *Girella punctata* GRAY T.L. 20.0mm

なお本種の産卵生態については水戸 ('57)[30] の報告があり，また全長 11.4mm の稚魚について中村 ('34 b)[31] の報告がある。本種の幼稚魚に関しては内田・その他 ('58)[45] の報告の中で，メジナの仔稚魚の内に疑問のまま報告している様に，同属に含まれる *G. melanichthys* クロメジナ及び *G. mezina* オキナメジナとの異同を明らかにした報告がないが，本種の幼稚魚が北海道南西部海岸附近の岩礁海岸及び藻場地帯に現われる事はその一点について報告した小林・鷲佐・高杉 ('58)[40] の報告中にも述べた様に，7月頃より全長20mm前後のものが磯辺に現われ，10～12月にかけて岩礁帯の藻場内で全長50～90mm 前後のものが見られる。これ等の大部分は殆んど体側も完成し明らかに本種と同定する事が出来る。その後沿磯附近の水温が10°C以下になる1月以降はこれ等の幼魚を見る事が出来ない。なおこれ等の標本に基く形態その他に関しては別に述べる予定であるが，以上の事等から考察してこの標本は *G. punctata* メジナの稚魚と同定出来る。

Gobiidae　クモハゼ科

Rhinogobius gymnauchen (BLEEKER)　ヒメハゼ

本種は全長89.0mmの1個体の成魚のみが汐目上の採集点1において得られた。

Aluteridae　カワハギ科

Stephanolepis cirrhifer (TEMMINCK & SCHLEGEL)　カワハギ

本種は全長4.5～8.5mm の4個体が汐目上の採集点1において得られた (Fig. 6)。本種の稚魚については内田 ('27)[44] 及び内田・その他 ('58)[45] が近縁種の稚魚と共にその形態を明らかにし，藤田 ('55)[4] は人工授精による卵及び稚魚について報告している。また対馬暖流開発調査によって対馬海峡水域における本種の稚



Fig. 6. *Stephanolepis cirrhifer* (Temminck & Schlegel) T.L. 8.5mm

魚の出現状況が明らかにされ，北部日本海水域では 8～10月の間に北海道南部以南の水域に全長 4～64mm のものが出現する事が報告された。

Tetraodontidae フグ科

Fugu sp.

本種の稚魚は全長 12.1mm (D. 13; A. 13; P. 15) の1個体が汐月上の採集点1において得られた (Fig. 7)。*Fugu* トラフグ属の卵及び稚魚の形態その他については中村 ('35 a)[32], Uno ('55)[50] の他，藤田

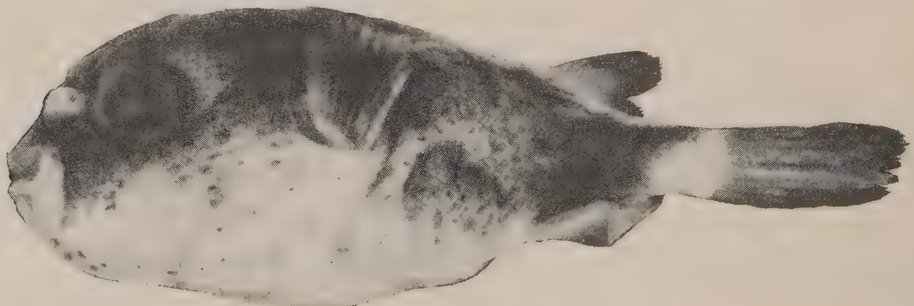


Fig. 7. *Fugu sp.* T.L. 12.1mm

('56 a[5], b[6]), 藤田・上野 ('56)[7], 庄島 ('57)[41] 及び内田・その他 ('58)[49] によつて逐次明らかにされ，既に明らかにされた種類に関しては近縁種間の幼期の検索も作製されているが，未だ不明種も多く，特に北部日本海に主として見られる *Fugu vermicularis porphyreus* (Temminck & Schlegel) マフグに就いては未だ報告がないので，本報告においては種名不明のまま記載する。

Scorpaenidae フサカサゴ科

本科に属するものは以下に述べる様に，*Sebastes* メバル属に含まれる4種が汐月上の採集点1において得

られ，その個体数も171個体でイトヨに次いで多数出現している。

従来メバル属の稚魚に関しては *S. schlegelii* クロソイ及び *S. pachycephalus* ムラソイの稚魚に関する Inaba[31]（'31 a）の報告，ムラソイの稚魚に関する中村[33]（'35 b）の記載の他 *S. inermis* メバルについて内田[17]（'43）の報告があり，また南樺太・南千島水域からの資料に基いて，Казанова[20]（'59）の報告中に *S. taczanowskii* エゾメバル及びメバル属2不明種の記載がある。然し未だ本属の種類の殆んどについてその幼稚魚形態は明らかにされていない。

Sebastes taczanowskii (Steindachner) エゾメバル

本種の稚魚は全長17.0～32.9mmの39個体が得られた。それ等の内主なる2個体について外形の概要を示すと下記の通りである。

a．全長21.0mm；体長16.9mm；D.XIII,14；A.III,7；P.15；鰓耙数11＋25；頭長は体長の2.60分の1；体高は体長の4.69分の1；尾柄高は頭長の3.82分の1。

体色は淡褐色で，横縞は無く，背鰭棘部外縁が稍々濃い黒色で縁取られている。

b．全長31.7mm；体長26.2mm；D.XIII,14；A.III,6；P.16；鰓耙数12＋27；頭長は体長の2.70分の1；体高は体長の4.03分の1；尾柄高は頭長の3.73分の1。

体色は淡褐色で，背部には明瞭な5条の横縞があり，背鰭に侵入する。また背鰭棘部外縁は稍々濃い黒色で縁取られている（Fig. 8）。



Fig. 8. *Sebastes taczanowskii* (Steindachner) T.L. 31.7mm

なお脊椎骨数（下尾軸骨を含む）は22個体についてX－rayにより観察した結果は25個のもの10個体，26個のもの12個体であつた。

Sebastes pachycephalus (Temminck & Schlegel) ムラソイ

本種の稚魚は全長21.0～25.6mmの7個体が得られた。

全長25.6mm；体長20.6mm；D.XIII,13；A.III,6；P.17；鰓耙数8＋18；脊椎骨数（下尾軸骨を含

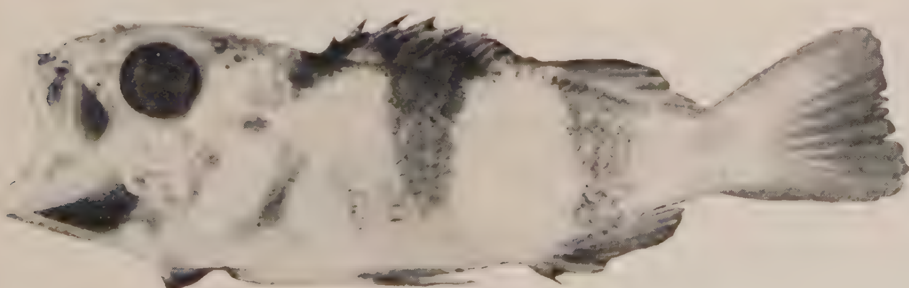


Fig. 9. *Sebastes pachycephalus* (Temminck & Schlegel) T.L. 25.6mm

む）26；頭長は体長の2.57分の1；体高は体長の3.32分の1；尾柄高は頭長の3.63分の1。

体色は淡黄色で，巾広い黒色の4横帯が有り，その第1帯は項部に，第2帯は背部では背鰭棘条部に侵入し，また腹側にわたる。第3帯は背鰭軟条部に僅かにかかり，臀鰭に侵入する。そして第4帯は尾柄部に在る。なお腹鰭の基部に近い約半分は黒い（Fig. 9）。

本種については前述の様に，中村（'35 b）[33]によつて報告されており，今回の標本も上記のように中村（'35 b）[33]の記載とよく一致する。然し本種は松原（'43）[22]によつて *S. p. nudus* オウゴンムラソイ，*S. p. pachycephalus* ムラソイ，*S. p. nigricans* ホシナシムラソイ及び *S. p. chalcogrammus* アカブチムラソイの4亜種に区別されており，今回の標本がそれ等の何れの型に属するかは判別し難い。

Sebastes sp. A.

本種の稚魚は全長13.0〜25.6mmの74個体が得られた。主なもの2個体についてその外形の概要を以下に記す。

a．全長17.9mm；体長14.4mm；D.XIII,12；A.III,7；P.15；鰓耙数9＋22；頭長は体長の2.82分の1；体高は体長の4.36分の1；尾柄高は頭長の3.00分の1。

b．全長25.1mm；体長20.2mm；D.XIII.12；A.III,7；P.16；鰓耙数11＋25；頭長は体長の2.85分の1；体高は体長の3.88分の1；尾柄高は頭長の3.08分の1（Fig. 10）。

体色は何れも淡黒色で，腹面では次第に淡色となり，特に縞は無く，各鰭は何れも無色である。

なお脊椎骨数（下尾軸骨を含む）は25個体についてX－rayによる計測では，25個のもの1個体，26個のもの6個体，27個のもの17個体及び28個のもの1個体であつた。

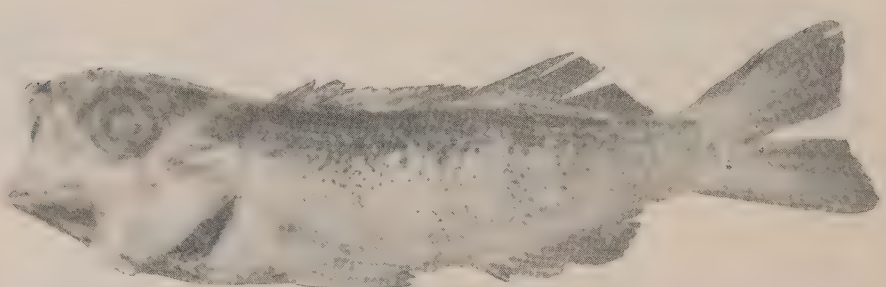


Fig. 10. *Sebastes sp.* A. T.L. 25.1mm

本種の稚魚は一見前述の *S. taczanowskii* エゾメバルの稚魚に似ているが，鰭条数，鰓耙数及び脊椎骨数等から *S. steindachneri* ヤナギノマイに近似のものと考えられる。

Sebastes sp. B.

本種の稚魚は全長15.7〜30.7mmの52個体が得られた。主なもの2個体についてその外形の概要を以下に記す。

a．全長15.7mm；体長12.4mm；D.XIII,12；A.III,7；P.18；鰓耙数6＋18；頭長は体長の2.63分の1；体高は体長の3.75分の1；尾柄高は頭長の3.91分の1。

体色は淡褐色で背側は稍々黒く，尾部体側中央が稍々黒いが，体には特に縞は無い。鰭は何れも無色である。

b．全長29.3mm；体長23.8mm；D.XIII,12；A.III,7；P.18；鰓耙数8＋18；頭長は体長の2.67分の1；体高は体長の3.66分の1；尾柄高は頭長の3.42分の1。

体色は淡褐色で，体側の項部に1条，背鰭棘条部に2条，背鰭軟条部に1条，尾柄部に1条，計5条

の不規則な淡黒色の横縞があり，何れも腹部に行くに従つて乱れて消える。これ等の横縞の背鰭棘条部のものは鰭に侵入し，且つ棘条部の外縁は巾広く黒く縁取られるが，他の鰭は何れも無色である(Fig.11)。

なお脊椎骨数（下尾軸骨を含む）は9個体について，X－rayによる計測では何れも26個であつた。

本種の稚魚は前述の *S. pachycephalus* ムラソイの稚魚とは体色及び体側の横縞等の差異で識別され，鰭条数，鰓耙数及び脊椎骨数等から ***S. trivittatus*** シマゾイまたは ***S. nivosus*** ゴマゾイに近似の種類と考えられる

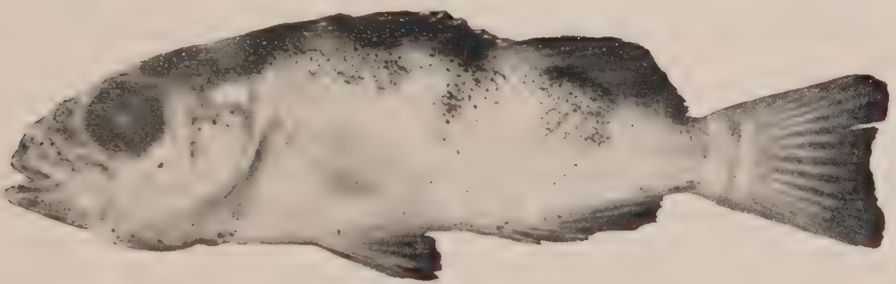


Fig. 11. *Sebastes sp.* B. T.L. 29.3mm

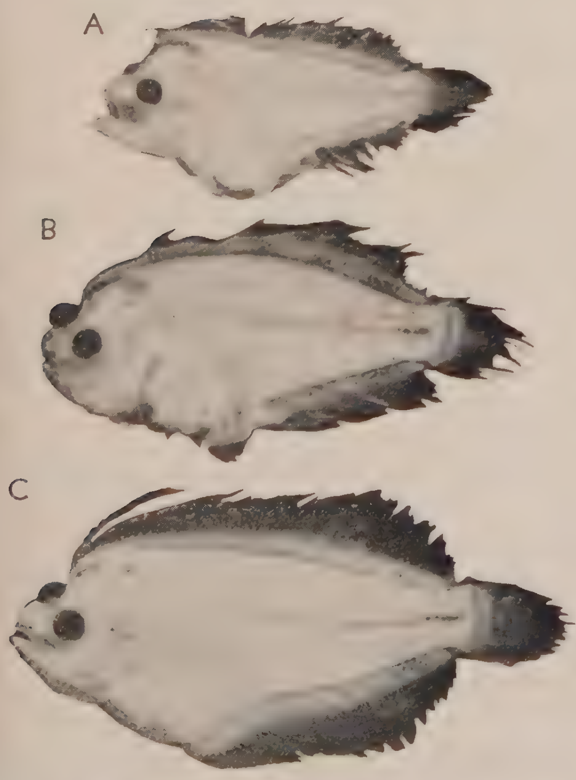


Fig. 12. *Paralichthys olivaceus* (TEMMINCK & SCHLEGEL)
T.L. A. 8.0mm, B. 9.0mm, C. 11.0mm.

Bothidae　ヒラメ科

Paralichthys olivaceus (TEMMINCK & SCHLEGEL)　ヒラメ

本種の稚魚は汐日の東側の採集点4において3個体と，汐日上の採集点1において1個体，計4個体全長 8.0～11.0mm のものが得られた。以下これ等のものについて，その外形の概要を全長11.0mm のものを中心として述べると下記の通りである

なお本種の稚魚については内田('58～'59)[48]によつてその変態期のものが示されており，今回の標本はそれと殆んど同じ成長段階のものである。

a．全長11.0mm; 体長9.0mm; D.74; A.55.

背鰭の第 2，3，4，5条は伸長し，その長さはほぼ頭長に等しい。右眼は背中線にかかるが，眼球は右側に偏つている。

黒褐色素は左側の背鰭及び臀鰭の基底に1列に並ぶ他，腹部及び尾柄近くに散在し，また背鰭及び臀鰭の外縁に比較的多く散在する。また背鰭の伸長した鰭条には特に色素が多い。右側にも左側同様に色素が見られるが，左側に比較して極

めて少い。(Fig. 12. C)

b. 全長約 9.0mm

右眼は背中線にかかるが，眼球は右側に偏つている。色素の分布状態は全長11.0mmのものよりむしろ密で，背鰭基底部附近に5個所，臀鰭基底部附近に3個所の濃密部がある。(F g. 12. B)

c. 全長8.5mm

右眼は全く右側に在り，色素も余り出現せず，僅かに背鰭及び臀鰭の基底に1列に並ぶ他，頭部に数個明瞭に在るが，体側では極く僅かしか見られない。

d. 全長8.0mm

色素は全長8.5mmのものより一層少い。また背鰭第2，3，4条は長く伸びその先端附近に色素が並ぶ(Fig. 12. A)。

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噴火湾における定置網の研究
第4報　ブリ落網の二重落網について

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Studies on Fixed Nets in Funka Bay
4. A study on the efficiency of the secondary bag nets for catching Yellowtail

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Abstract

The writers have carried out a study on the efficiency of secondary bag nets for yellowtail in Funka Bay since 1955. The conclusion is as follows,

1. Most of the secondary bag nets which have been employed at Funka Bay have the construction of purse style as shown in Fig 4.
   The shape of a net of saran is more steady and the ratio of falling into a trap is greater than those of manila twine.
2. It is more effective for the purpose of improving the ratio of yellowtail falling into a trap that the depth of the HANTATEAGE and the TATEAGE is cut more shallow to some extent than the depth of water and that the hanging of original bag net be kept comparatively shallow.
3. The position at which the funnel net is attached to the secondary bag net employed at Funka Bay is appropriate at four to seven meters below the surface from the standpoint of construction of secondary bag net, condition of secondary bag net to be attached to shape ropes, behavior of yellowtail which came into the net, and moreover for the sake of convenience of operation.
4. When the current flows from the direction of heart part, the greater part of yellowtails fall into the secondary bag net attached to the TATEAGE rather than into other 1-2 secondary bag net.

When one makes a diagram of a yellowtail trap net which is to be set in waters where the net shape is steady owing to the rather weak current, the writers believe it is effective to lessen the total volume of the bag net in order to make rapidly and securely the yellowtail that have come into the bag net pass on into the secondary bag net.

1　緒　　　言

噴火湾における定置網漁業（主として落網）は，最近不振状態を続けているが，しかし，ブリ・マグロ・サバ・イワシ・イカその他の漁獲を目的とする本網への期待は甚大なものである。

そもそも，この落網は一定期間，一定場所に敷設されるものであり，その漁場条件としては，これら目的魚の回遊経路の究明，敷設場所における自然的諸条件等があげられるが，これらの条件を満足させるためにも漁獲効果を最大にしなければならぬ。

すなわち，目的魚の習性の究明は勿論のこと，本網の敷設海域の精密な現場調査，それに伴う合理的漁網設計が必要である。

落網の一般構造から見て，一度箱網に落ちた魚の逃逸，波浪による網の動揺，網成りの変形等で逃逸する

ことがしばしばある。特にブリを目的とした落網では，この点が顕著であるが故にこれらの逃逸を防ぐため箱網にさらに狭い漏斗を通して別の強靱な仕立ての小袋網の中に陥入し漁獲することが必要である。この小袋網がすなわち，二重落網（きんこ）である。

当教室では昭和30年以来，噴火湾にて使用される二重落網につき調査を進めておりここにその大綱の一端を報告する。

2 調査方法

昭和30年以来，森・尾白内・砂原・掛澗の各海区および地理的にも外洋に面している，川汲・尾札部・木直・古部の各海区に敷設される，ブリ・マグロ専用大型落網およびブリ兼用小型落網中，二重落網を布設しているものについて調査した。なお，調査期間は毎年6月〜11月までとし，この期間中，昭和33年よりは特定海区での定期海洋観測をも併せ行い，これら海区の敷設網の各局部の網成り等の点をも究明すべくその準備を進めていたが，観測続行中に計測器類の故障のため中止した。なお，この観測に使用した計測器は次の如し。

(1) 電気温度計　　　CT—5型　（応用電子工業株式会社）

(2) 電気流向流速計　CM—2型　（東邦電探株式会社）

3 調査結果

ブリは北海道周辺海域で，ほぼ例年的に，かなり大量に漁獲されており，また，最近は対馬暖流調査に伴い，ブリ回遊経路にも考察を加えられ，北海道渡島海区漁場では，晩夏から初秋の候には相当量漁獲されている。[1]特に噴火湾では従来，ブリ・マグロ専用落網が無く主として，マグロ・イワシ・サバ等と混獲されていた程度であつたが，ここ4〜5年前より合成繊維漁網の定置への普及が目立ち，これらを使用したいわゆるブリ・マグロ専用落網（二重落網付き）が出現して以来，優秀な漁獲成績をあげている。

ブリ落網の漁場水深は，30〜75m位の範囲にあり，普通は45〜60.6mの漁場が非常に多い[2]が，当海区の敷設水深は，18.2〜57.6mの範囲であり，一般に水深が増すに従い自然，敷設網も沖に出て，かつ，網型も大きくまたそれだけ潮流の影響も大きくなることは当然である。すなわち，各敷設場所における波浪の大小潮流の強弱，これに伴う網が受ける抵抗，衝撃の限界等の調査も当然必要である。

ブリ定置網では網型も大きく，極く弱い潮流時でも，その抵抗が非常に大きく，各局部の網成りの変形も著しくなるため，漁場としてはなるべく潮流の緩漫な処を選定するのが理想であろう。また，余剰浮力の点についてもこれら網地の資材，網自体の構造および操業できうる流速の限界等を考え決定しなければならぬ。すなわち，かような点とブリの習性とを合せ考えて，一度箱網に陥入したブリをその後，網の変形，沈降等によつて逃逸させることなくできうる限り速かに袋網に陥れ，一度この中に入つたブリはいかなる海況のもとにあつても，これを完全漁獲するため二重落網を布設するのである。

当海区では従来，相当量のブリの来遊を見ながら芳しい漁獲をあげ得なかつたのは，ブリの習性の研究は勿論のこと，これらの網の特殊構造の研究に乏しかつたことにも起因する。

一般に二重落網（きんこ）を箱網部に取付ける方法に二通りある。[3]すなわち，箱網と袋網とを連結する個所に登網と同様な構造の登網をつけるもの，今一つは箱網と袋網とを連結する個所を角筒にするものとであるが，当海区の二重落網の取付構造は全部後者に属するものである。

第1図は森海区で使用する二重落網の附設図である。すなわち，二重落網を半建揚部の沖，丘側および建揚部の三箇所に附設するものであるが，漁況によりいずれかを欠くこともある。なお，本網の上部には硝子玉で浮力を与え，かつ「はつぴやく」を使用し，あたかもニシン枠網の如き構造にしているが，実際にはこの式の構造は操業上誠に不便である。

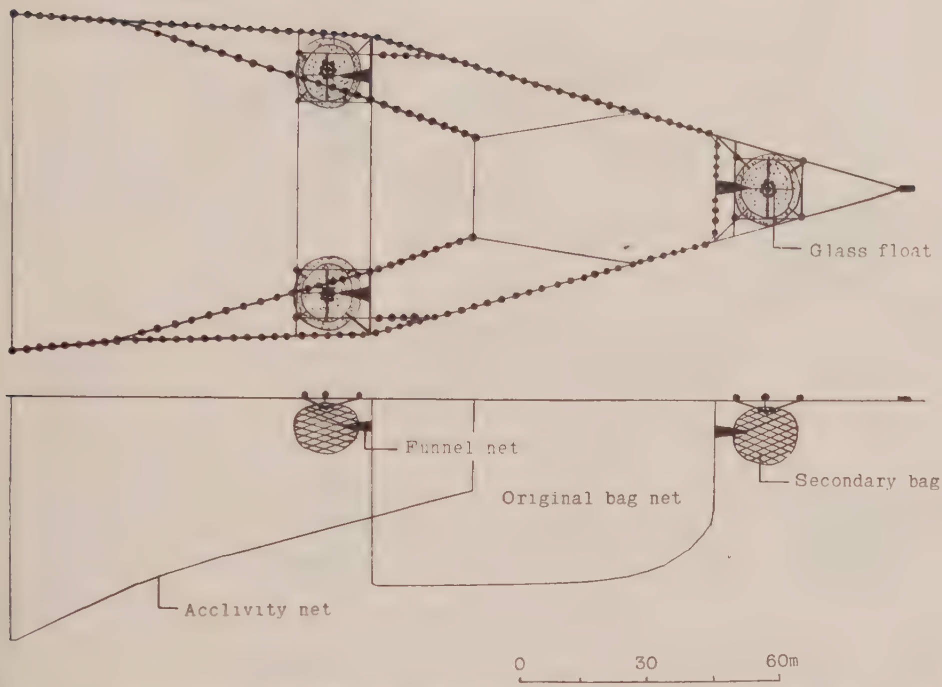


Fig. 1. Diagram of the secondary bag nets attached to the HANTATEAGE and the TATEAGE which are employed in Mori region of Funka Bay

第2図は当海区で使用する二重落網および漏斗網の展開図であり，第1表はこれらの材料見積表である。

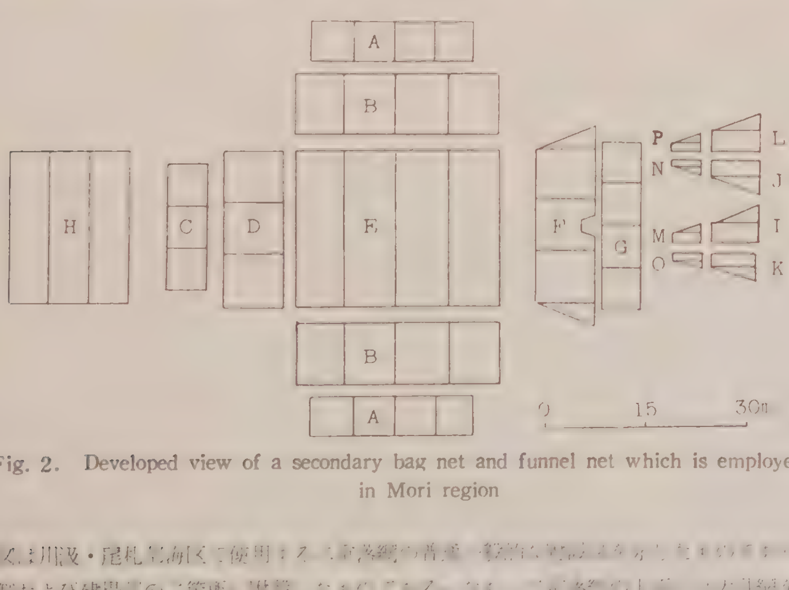


Fig. 2. Developed view of a secondary bag net and funnel net which is employed in Mori region

次に第3図は川汲・尾札部海区で使用する二重落網の普通一般的に[illegible]ものであるが，本網を[illegible]建揚網の沖側および建揚網の二箇所に附設したものである。[illegible]二重落網の上部には大目網を張らないで，上縁は巾着網の結括式構造に仕立てている。（大目網は浮力[illegible]

Table 1. Estimated material of a secondary bag net and funnel net such as employed in Mori region of Funka Bay

Mark	Material of thread	Varieties of thread	Mesh size	Number of mesh	Length of net	Number of net	Use of net
A	Manila twine	6g(1.5m)	12.1cm	50	6.1m	4×2	Longitudinal direction
B	〃	7.5g	7.6cm	100	9.1m	4×2	ditto
C	〃	6 g	12.1cm	50	6.1m	3	ditto
D	〃	7.5g	7.6cm	100	9.1m	3	ditto
E	〃	7.5g	7.6cm	100	22.7m	4	Transverse direction
F	〃	7.5g	7.6cm	100	9.1m	3.5	Longitudinal direction
G	〃	6 g	12.1cm	50	6.1m	4	ditto
H	〃	6 g	12.1cm	50	22.7m	3	Transverse direction
I	〃	7.5g	6.1cm	50	7.6m	1.5	Longitudinal direction
J	〃	7.5g	6.1cm	40	7.6m	1.5	ditto
K	〃	7.5g	6.1cm	40	6.1m	1.5	ditto
L	〃	7.5g	6.1cm	50	7.6m	1.5	ditto
M	〃	7.5g	6.1cm	25	4.5m	1.5	ditto
N	〃	7.5g	6.1cm	20	4.5m	1.5	ditto
O	〃	7.5g	6.1cm	20	4.5m	1.5	ditto
P	〃	7.5g	6.1cm	25	4.5m	1.5	ditto

(Remark : Hopping 0.2～0.34)

第4図はこれらの展開図，第2表はこれらの材料見積表である。

全般的に見て，噴火湾において使用される二重落網の構造は，第4図の如き構造のものを殆んど使用しており，また，資材の点でも最近マニラトワインからサランに変りつつありˮ これは使用中における網糸の抗張力の低下がマニラトワインより少く，糸質が細く水切れが良く，網の沈降状態等の点から見ても，サランを使用した時の方が網成りが良好かつ，漁獲も優秀である。

次にこの二重落網の有効性であるが，これは箱網の構造および二重落網の漏斗網の取付位置，袋網の垂下状態（位置）等により多分に左右されると考えられる。

第3表は上記調査海区に敷設される網中，最深漁場から最浅漁場の範囲にある数種の網についての主要局部の高さ（深さ）を示した。すなわち，この表から見ても解る如く，

1 水深に対して運動場の丈は一様に余裕を多くし，多少の網の吹かれに対しても入網魚の網裾よりの逃避を防ぎ，一度運動場に入つたブリの箱網への陥入率を良好にしている。

2 半建揚の高さは14.2～48mの範囲内で水深の72～83%，また建揚の高さは，それより幾分短目に仕立てており，半建揚は比較的浅い方が箱網の構造から見ても，この箱網の敷の垂下の度合が少く，一度箱網に入つたブリの行動範囲が狭少され，その後網をつたわり，かつ，迷路を求めつつ，袋網すなわち，二重落網に落ち易い。

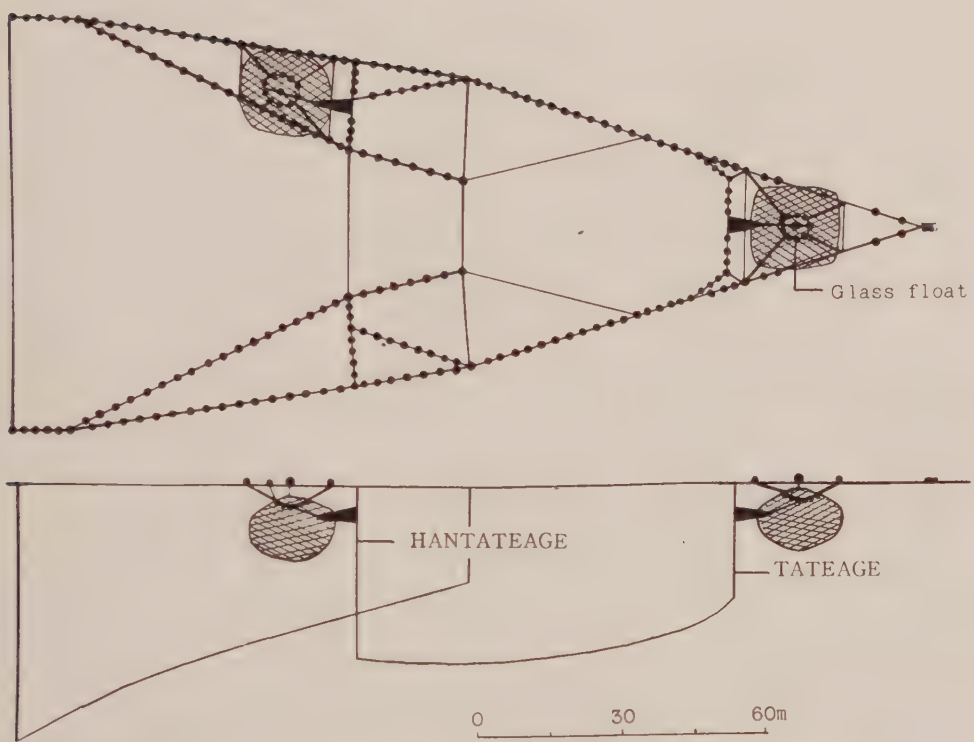


Fig. 3. Diagram of the secondary bag nets attached to the HANTATEAGE and the TATEAGE which are employed in Kakkumi region

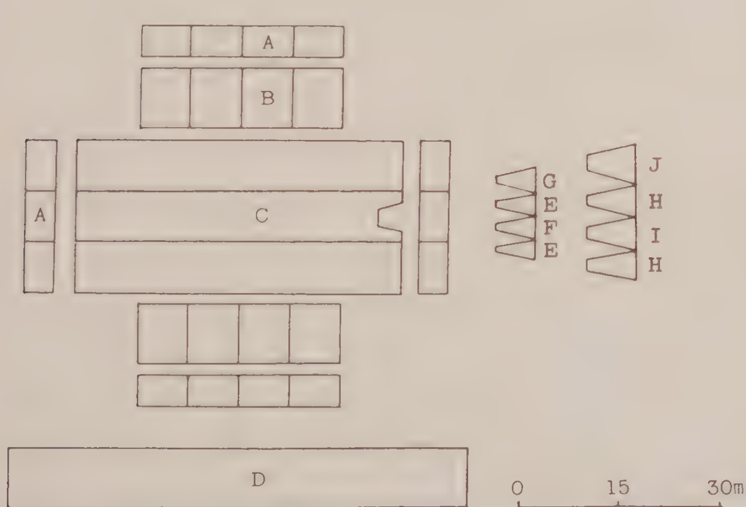


Fig. 4. Developed view of a secondary bag net and funnel net which is employed in Kakkumi region

Table 2. Estimated material of a secondary bag net and funnel net such as employed in Kakkumi region

Mark	Material of thread	Varieties of thread	Mesh size	Number of mesh	Length of net	Number of net	Use of net
A	Saran	1000 D 36Yarns	7.6cm	100	4.5m	14	Longitudinal direction
B	〃	44Yarns	7.6cm	100	9.1m	8	ditto
C	〃	44Yarns	7.6cm	100	48.5m	3	ditto
D	〃	36Yarns	9.1cm	100	68.2m	1	Mouth net
E	〃	36Yarns	7.6cm	40〜15	6.1m	2	Longitudinal direction
F	〃	36Yarns	7.6cm	60〜15	6.1m	1	ditto
G	〃	36Yarns	7.6cm	65〜15	6.1m	1	ditto
H	〃	36Yarns	9.1cm	50〜35	7.6m	2	ditto
I	〃	36Yarns	9.1cm	50〜45	7.6m	1	ditto
J	〃	36Yarns	9.1cm	65〜50	7.6m	1	ditto

Table 3. Height of main parts of nets studied

Sign of investigated net	Depth of net's mouth	Height of heart net (shore)	Height of heart net (offing)	Height of HANTATEAGE	Height of joining point of acclivity net	Height of funnel net
A	57.57m	66.66m	69.0m	48.0m	30.3m	7 m
B	53.0 m	57.57m	62.1m	38.0m	25.8m	4.6m
C	45.45m	50.0 m	51.5m	35.0m	24.0m	4.0m
D	18.18m	21.21m	22.0m	14.2m	9.0m	

3 外登，内登の接合部の海面迄の高さは9〜30.3mの範囲で，半建揚の高さの63〜69%であり，これより深くした場合は勾配がゆるやかになり過ぎ登網および箱網の目的を達せず，逆に浅くし過ぎた場合は勾配が急勾配になり箱網への陥入率が悪くなる。一般に当海区での登網勾配は13〜16度位が多く，中には18〜19度，またそれ以上のものもある。（まぐろその他を目的とした場合）

4 二重落網の漏斗網の取付位置は箱網の大きさ等により多少の差があるが，大体海面下4〜7mの範囲内にあり，これより浅い場合は潮流による浮子方の沈降等で漏斗口の効果を減じ，また，潮流の支障のない海面平穏な時でも，二重落網の構造からして海面に浮上し，本網中に落ちたブリの行動が落着かず網身体の安定感を欠くことになる。また深くした場合は本網の構造および各型網への取付状況から見ても操作が困難になる。二重落網は第1図および第3図の如く各型網により支へられ，かつ，垂下しており，締括網をといた時，その下方についている硝子玉の部分が自然に浮き上る程度が適当である。

5 運動場の方向よりの一方的な潮流時において箱網に取付けられるこれら数箇の二重落網への陥入率を見た場合，建揚部に布設した二重落網が最良であり，次いで半建揚部の沖，丘の二重落網の順であつた。

4　総　　　括

以上の調査結果より見るに

1　噴火湾において使用される二重落網は，第4図の如き締括式構造のものが多く使用され，かつサランの網成りは，マニラトワインより良好であり陥入率も優秀である。

2　半建揚および建揚の丈を水深より或る程度浅く，箱網の敷の垂下の度合を少くすることは，ブリの二重落網への陥入率を良好にする

3　当海区における二重落網の漏斗網の取付位置は，二重落網の構造，取付状況，陥入したブリの行動および本網の操作等の面から見て海面下4〜7 mの範囲が適当である。

4　運動場の方向よりの潮流時においては，建揚部に布設した二重落網への陥入率は他の1〜2の二重落網への陥入率より遙かに良好である

5　潮流が比較的弱く，網成りの変形の少い海区でのブリ専用落網（二重落網付）の仕立てを考えた場合一度箱網に陥入したブリを，さらに，より速かに，確実に二重落網に陥入させるために，今少し箱網の容積を縮小して位立てた方が効果的と思う。

5　結　　　言

噴火湾における定置網の研究の第4報として，ブリ二重落網の構造およびこの有効性の一端を述べた。なお，当海区における潮流は，いわゆる下り潮（4〜8月・湾口より湾奥への潮），上り潮（9〜3月・前者の反対の潮）であり，この下り潮により操業上，困難をきたすこともあるが，主として上り潮による定置網の被害が甚大である。今後，漁場調査，定期海洋観測を続行し，これら定置網の構成，固定等の点も考究しなければならぬ。

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水産物の機械的性質
1 スルメの引張強度と伸び

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Mechanical Properties of Marine Products
I. Tensile strength and elongation of dried squid

Tetsuo OHKAWA

Abstract

Today in Japan, the judgement of dried squid grade is still dependent upon only the observing eye and nose of the inspector, for size, gloss and smell; thereby, the products are classified into five grades. On this experiment, the relations between the classification and the mechanical properties (tensile stress and strain) of dried squid meat are studied.

The dimensions of test materials are shown in Table 1. The test pieces were cut in 5 × 50 mm in size and pulled by the testing aparatus, shown in Fig. 2. Figs. 3 – 7 show the stress-strain diagrams of these examined products.

According to these diagrams, the difference of the tensile strength is invisible but the strain is different in each class. The strain of the higher classes is evidently larger than the lower (See Figs. 6 and 7).

Because of this, if it is desireable to classify the product by mechanical properties, it should be decided by testing the strain not the strength.

現在市販されているスルメは1等品から5等品まで5段階にわけられているが，これは主として色沢や葉揃いなどの肉眼的観測及び芳香により級別されている。この実験ではこれら級別されている製品の抗張力，歪みなどの機械的性質からその妥当性が裏付け出来ないかと考えて試みたものである。

実験に際して，種々御指導を賜つた小林喜一郎教授，並びに試料入手に斡旋の労をとられた北水試奥田・佐藤両技師に謝意を表する。

1 実　　験

試料は秋採スルメと称するもので同一時期（昭和34年10月）に，同一地区（函館市郊外下海岸）で作られた市販品を取上げ，1～3等品は任意に5枚宛選び，4～5等品は同様に10枚選出した。

Table 1. Dimensions of experimental materials

Grade	Wt. / Leaf	Dimensions		
		Width	Mantle length	Arm length
1	68.6 g	105mm	219 mm	270 mm
2	64.6	112	216	295
3	53.4	110	192	267
4	45.3	106	184	249
5	40.3	121	195	227

Table　1の試料を，その胴部下端から30mmの処を体軸に直角方向に巾5 mm長さ約50mmの試片を4片とり，これを体軸に直交方向の強度を測定する試片とした　この試片の中央部20mmの個所て荷重　伸びの関係を測定した。[1)]

更にそのすぐ上部に体軸に平行方向に同様寸法の試片を4片とり，体軸に平行方向の荷重一伸びの関係を測定した。

実験装置の概略はFig. 1に示す　荷重はFig. 2の如く一定水位の水を注入することにより時間の函数とし

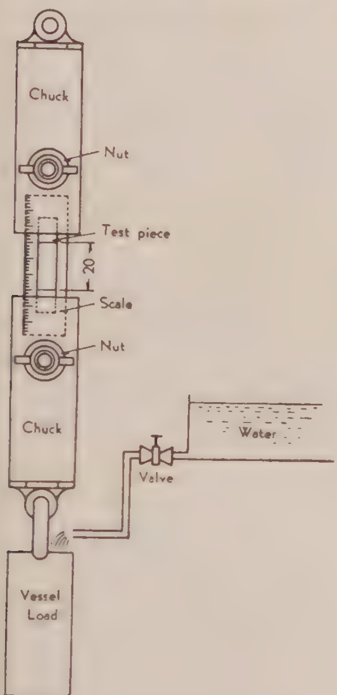


Fig. 1.　Schematic figure of experimental apparatus

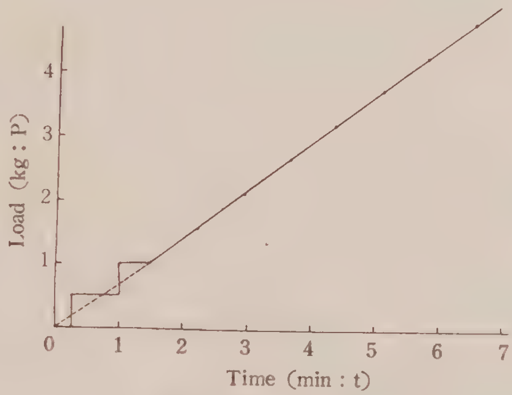


Fig. 2.　Load-time diagram

荷重 $P = \frac{2}{3}\ t$　　〔tは時間 (min.)〕(kg)

であらわされるような漸加荷重とし，このFig. 2線図の各黒丸点毎にその時の伸びを測定した。

Fig. 3は，体軸に直交方向の荷重と伸びとの関係を示す曲線であり，Fig. 4は体軸に平行方向のものを示す。

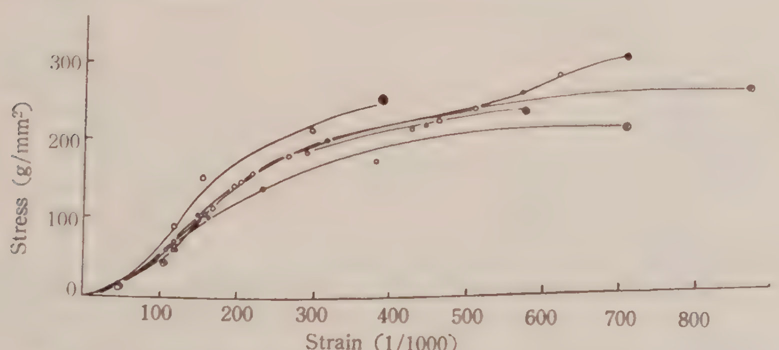


Fig. 3-1.　Stress-strain diagrams in right angled direction to mantle axis, Grade　1

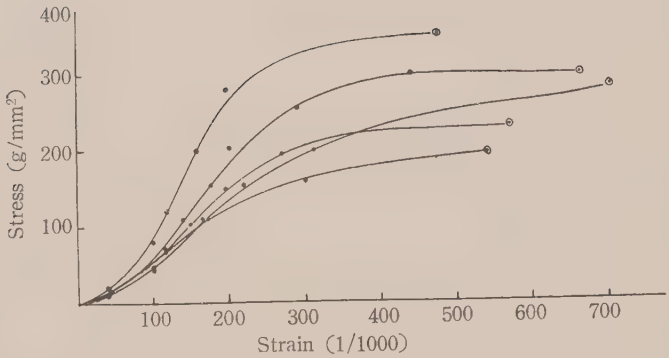


Fig. 3-2. Stress-strain diagrams in right angled direction to mantle axis, Grade 2

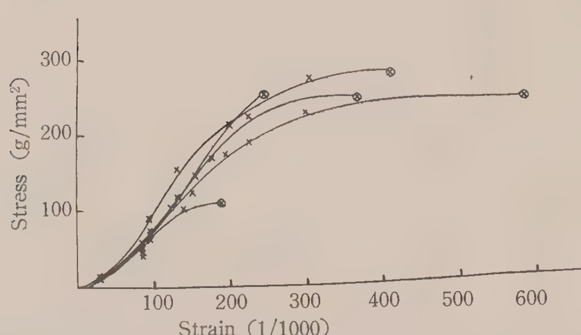


Fig. 3-3. Stress-strain diagrams in right angled direction to mantle axis, Grade 3

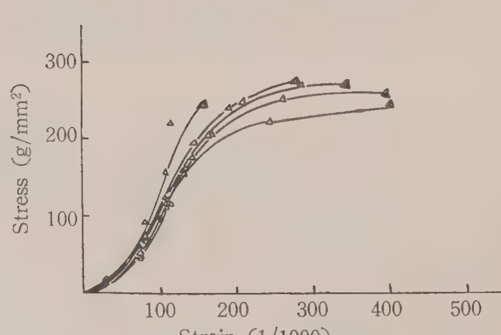


Fig. 3-4. Stress-strain diagrams in right angled direction to mantle axis, Grade 4

2 検 討

Table 1は，級別された試料についてその諸元を測定したものである。表中，巾と称するのはスルメを概略二等辺三角形と見做した時のその底辺に当る寸法である。これによると，下級品になるにしたがつて1枚当りの平均重量は軽くなつているし，またFig. 5には1枚当りの平均厚さをあらわす函数として，各級に対する1枚当り重量÷（巾×体長）を示しているが，これでみても下級品になるにつれてその厚さは薄くなつていることが判る。殊に5等品は雨イカスルメと称せられるものであつて，これは他に比して一段と薄くなつている。この差異も現在の級別判定の一つの要素になつている。

(1) 体軸直交方向の強さと伸び

上述の如く，等級が下がるにつれて肉が薄くなつているが，体軸に直交方向の抗張力を測定した結果を平均して一つにまとめてみると（Fig. 6），1等品から4等品まではその差はあまり認められない。5等品は雨イカであるせいか他に比してむしろ強い。

然し，歪みについては1等品と2等品の間には殆ど差はなく他に比して大きな値を示しているが，3等品から5等品になるにつれてその順に減少していることがはつきりあらわれている。然し，1等品と2等品とでは (Fig. 3 (1)と(2)) 1等品の方がまとまつている即ち品質に強度上の均一性があり，2等品の方がバラツキが多いといえる。

また，荷重に対して最高伸びのバラツキは下級品になるにつれて多いことが判る。然しこの場合でも下級品は重荷重に耐え得てもその歪みは級別順にしたがつて小さくなつている。

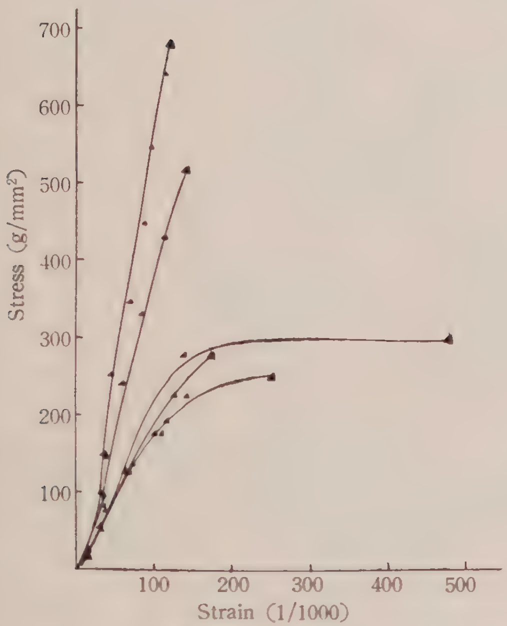


Fig. 3-5.　Stress-strain diagrams in right angled direction to mantle axis, Grade 5

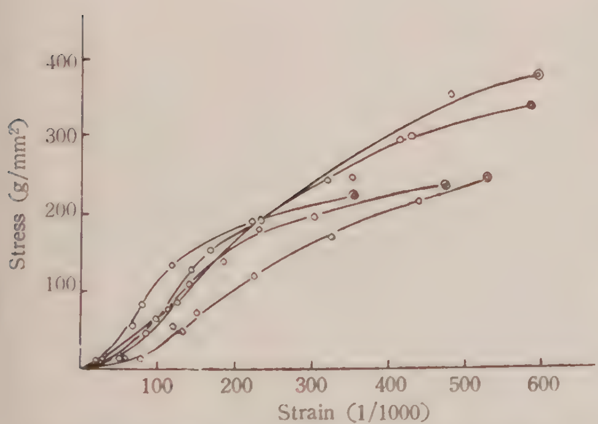


Fig. 4-1.　Stress-strain diagrams in parallel direction to mantle axis, Grade 1

⑵　体軸平行方向の強度と伸び

体軸に平行な方向の荷重—伸び（歪み）の関係はFig. 7に示す。この関係は定性的には体軸直交方向の関係と似ているが数値は小さく，各等級共全般的に相違する点は上級品といえども極限強さに近づいた時に，流伸が少なく従つて局部的狭搾が少なく，突如として破損することである。この理由はイカの肉組織は外套を環走しているらしい筋線維層と，その層間を表側から内臓側へ直走する筋線維層との2層から成ることに原因するものと思われる。[2)]

強さに関しては，1及び2等品は体軸直交方向のそれと差はみられなく値も揃つており，3 4 5等品に比して大きい数値である。3等品がこれにつぎ続いて4及び5等品が殆ど同値になつている。

歪みについては，5等品がその数値が体軸直交方向のものより僅か少さくなつているにすぎないが，他のものはかなり小さくなつており，その結果は1～5等品の順に明らかに伸びが小さくなつている。この上級品ほど伸びの大きいことは，上級品ほど肉質の延伸性の多いせいであろうと思う。実験中でも上級品ほど荷重中に表皮と肉質がはがれにくく，下級品になるにつれてはがれ易かつた。殊に5等品は肉質の伸びが少なくて，それが破壊されても表皮が残り表皮のみで荷重に耐えているような現象が起ることが時にみられた。

⑶　結　　　論

この秋採スルメの実験によつて，結論として云えることは，最初実験前に各等級別によつてその抗張力に差が生ずるであろうと予測していたことは間違いであることが判つた。

機械的性質を級別判定の資料とするならば，抗張力の大小によつて判定することは妥当でなく，むしろ歪みの大小，即ち伸びの大きい体軸直交方向の歪みの大小によつて級別することの方がはるかに確実である。後採スルメや夏採スルメについてもこのことが云えるかは今後の実験によることが必要である。

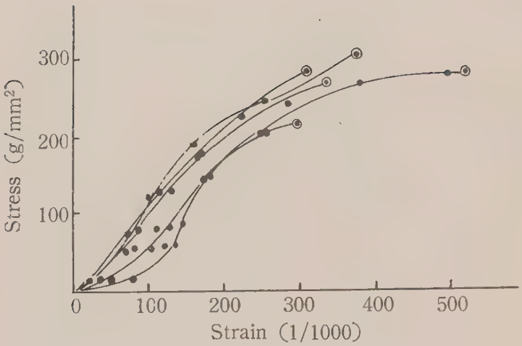


Fig. 4-2. Stress-strain diagrams in parallel direction to mantle axis, Grade 2

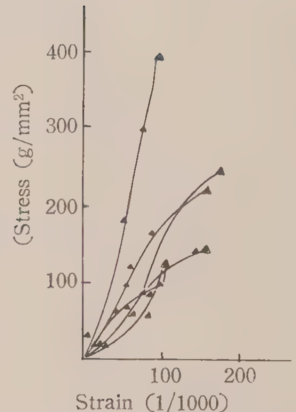


Fig. 4-5. Stress-strain diagrams in parallel direction to mantle axis, Grade 5

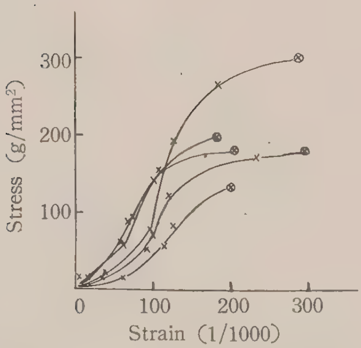


Fig. 4 3. Stress-strain diagrams in parallele direction to mantle axis, Grade 3

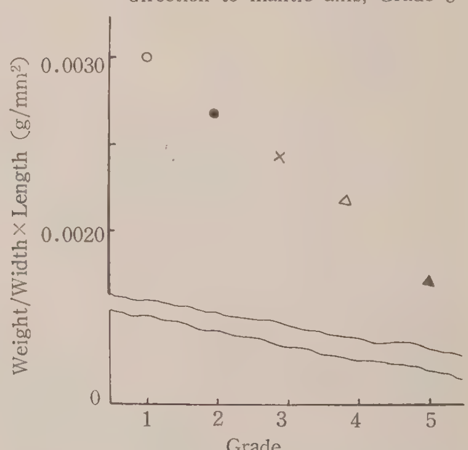


Fig. 5. Relation between weight and grade
○ : Grade 1 ● : Grade 2 × : Grade 3
△ : Grade 4 ▲ : Grade 5

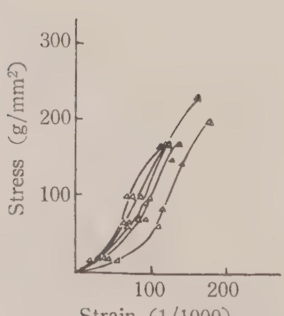


Fig. 4-4. Stress-strain diagrams in parallel direction to mantle axis, Grade 4

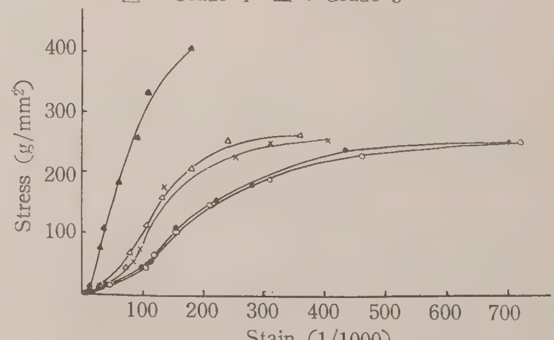


Fig. 6. Stress-strain diagrams in right angled direction to mantle axis
○ : Grade 1 ● : Grade 2 × : Grade 3
△ : Grade 4 ▲ : Grade 5

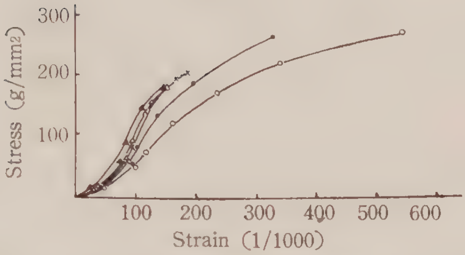


Fig. 7. Stress-strain diagrams in parallel direction to mantle axis
○ : Grade 1　● : Grade 2　× : Grade 3
△ : Grade 4　▲ : Grade 5

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石狩川河水の流出によつて湾内にできる潮目について

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On the Current Rip in Ishikari Bay caused by the Ishikari River flowing into that bay

Tsuneyoshi SUZUKI and Noritatsu SANO

Abstract

It is a matter of course to say that boundary zones take a very significant part in the formation of fishing grounds. The writers have carried out investigations on the form of the current rip in Ishikari Bay caused by the inflow of the Ishikari River in order to clarify the effect of a boundary zone by means of ultra sonic waves. The current rip shows the convergence line of two water masses. The one formed in Ishikari Bay is caused by the convergence of a bay stream which washes along the Shakotan peninsula and the estuarine water of the Ishikari River. This is classified into the form of a current rip. It is characterized by the wind direction into the following two types.

1) Type A due to a south wind. In this type, a nearly straight convergence line is formed at the left side off the estuary.
2) Type B due to a north wind. A convergence line is formed parallel to the coast line adjacent to the estuary.

As regards the reflection of supersonic waves around the current rip, the reflection loss was observed using an NS-300A type (200kc) fish finder produced by K.K. Sanken. The reflection of supersonic waves from the current rip seems to be due to the bubble layer caused by the collision of the two water masses; its reflection loss was ca. 60db. Flowing in of the river causes a water region in which the density differs greatly. The reflection loss amounted to 44-5db when the density in situ showed ca. $4.0^{g/l}$/m, and ca. 70db when the latter showed ca. $0.7^{g}/^{l}$/m. The reflection of supersonic waves from suspended matter may reasonably be caused by organic suspension brought down by the river water; its reflection was ca.45db when the extinction coefficient showed 0.55 and ca.80db when the latter showed ca.0.18. Concering the horizontal distribution of larvae and young fishes, writers were able to catch them in larger number at the current rip as compared with those caught at the stations neighboring the current rip. This fact suggests that the young fish might be gathered by the convergent current on the current-rip.

1　緒　　　言

潮境が漁場形成上非常に重要な役割りを演じていることは，今更言を俟たないが，筆者等は，現在超音波から見た潮境の問題を種々究明する目的をもつて調査中であり，先に“潮境の探知”という問題について，レーダー，航空機，音響探知機，肉眼等による探索方法について報告[1]したが，今回は，1958年7月より，1959年12月にわたり，石狩川河水の流出によつて湾内にできる潮目の形態，またその附近の超音波による反射状態，および稚魚分布の関連等について調査したので報告する。なおこの調査の一部は，1958年および1959年度の北海道科学研究助成金によつている。

2　潮目の分布状態

潮境は水塊の不連続面におこり，潮目は両水塊の流れの収斂部に生ずるものである。石狩川河水の流出に

よつてできる潮目も，流出する河水と，湾内を流れる湾流との収斂によりできている。北半球では一般に，海水の流動は，地球自転の偏向力のため，湾口から湾奥に向つて左側に外洋水か流入し，湾内でできた沿岸水は，左側を流出するのが通例であるが[2]石狩湾の状態も一般[3]的には，全くこれと同じであり，暖水系の外洋水が，積丹半島ぞいに浸入して，湾内に左旋流をおこさせているが，時として雄冬岬沖の冷水塊が湾口をふさぐが如く発達する年度があり，かような時は，暖流系の外洋水はこの沖合を流去してしまうので，湾内には右旋流が起るようになる[1]。Fig. 1は一般的状態における石狩湾表面の湾流模型図である。しかして1958，1959年度の調査中はこの一般的型に属する型であり，河口より沖合をみて，その左側にその収斂部が生じ，いわゆる潮目としての顕著なものがあらわれている。

次に石狩湾は，沖合に対し北西側が開けているため，北風系の風が吹くと沖合より湾内に向う吹送流が生じ，このため，低密度の河水は沿岸にそつて楔状に堆積されてしまい，そこに不連続面を生ずるため，風向によって潮目の分布状態が著しく異つている。

すなわち，（a） 南風系の場合は，河口の左側より沖合に向つて，ほぼ一直線の潮目があらわれる。

（b） 北風系の場合は，沖合からの吹送流のため，河水は沿岸に堆積されるので，沿岸にそつて，ほぼ平行な潮目があらわれる。

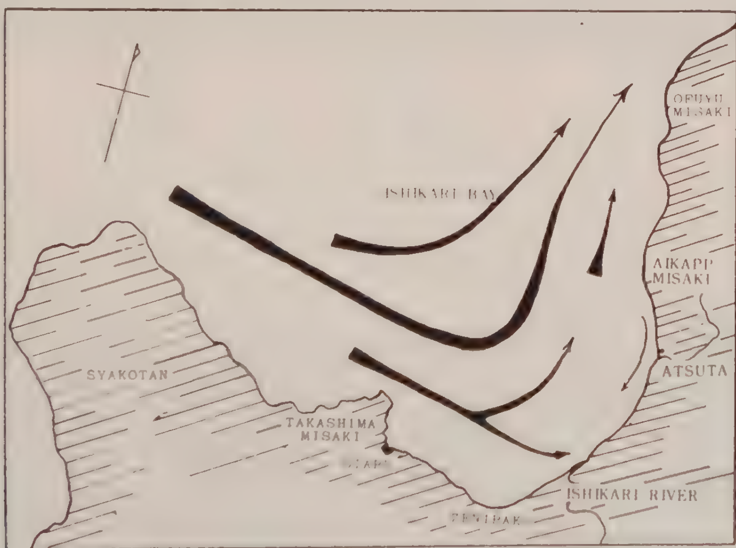


Fig. 1. Schematic representation of the hydrographic conditions of Ishikari Bay

Fig. 2 は1959年3月より12月にわたつて毎月1回～2回のわりで，航空機により観測した見取図で，これでわかる如く，南風系の場合は，大体風向にそつて沖合にほぼ直線的にでき，しかも風の比較的弱い時は，河口より見て，左側に1本著明な潮目ができるだけであるが，風が強くなると，河口の右側にも不明瞭ながらもう1本できる。これは，Fig. 22, 23でわかる如く，低密度の河水が湾内に流入すると，垂直安定度がよいので，沖合に拡散する場合特に風の影響を受けるものと思われる。

北風系の場合は，図の如く沿岸にほぼ平行にできている。

Fig. 3，4，5は南風系の場合の潮目の状態を航空機により撮影したもので，河口より沖合にほぼ一直線に収斂線が存在しており，Fig. 6，7，8は北風系の時の状態を写したもので，はつきりと沿岸に平行な線で境界線ができており，Fig. 9，10は南風系の場合の潮目を海上より写したものである。

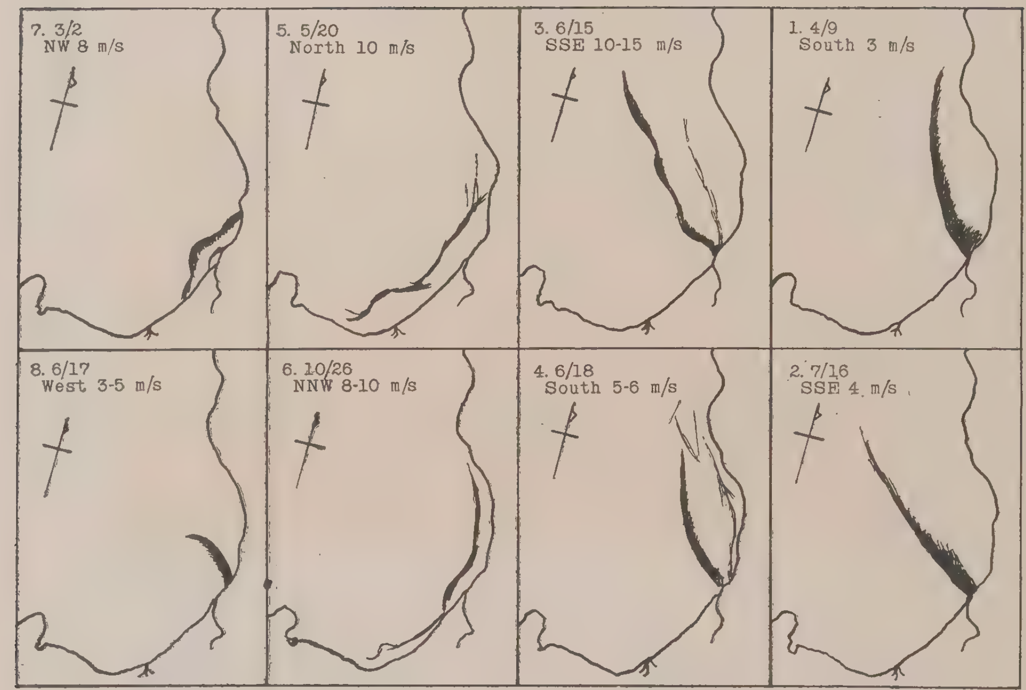


Fig. 2. Rough sketch of current rip in Ishikari Bay observed by airplane



Fig. 3.



Fig. 4.



Fig. 5.

Figs. 3-5. These photographs were taken from an airplane, indicating the current rip in Ishikari Bay when the wind blows from the south caused by the Ishikari River which flows into that bay.



Fig. 6.



Fig. 7.

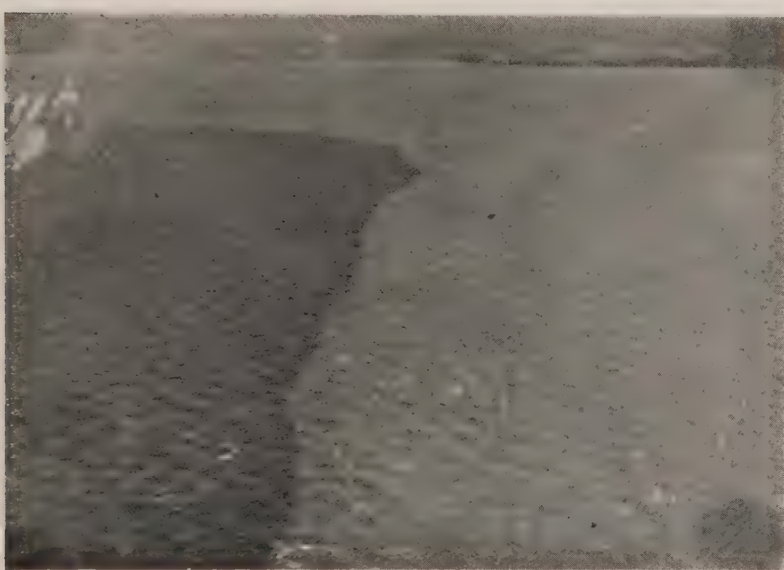


Fig. 8.

Figs. 6–8. These photographs were taken from an airplane to show the current rip in Ishikari Bay when the wind blows from the north.

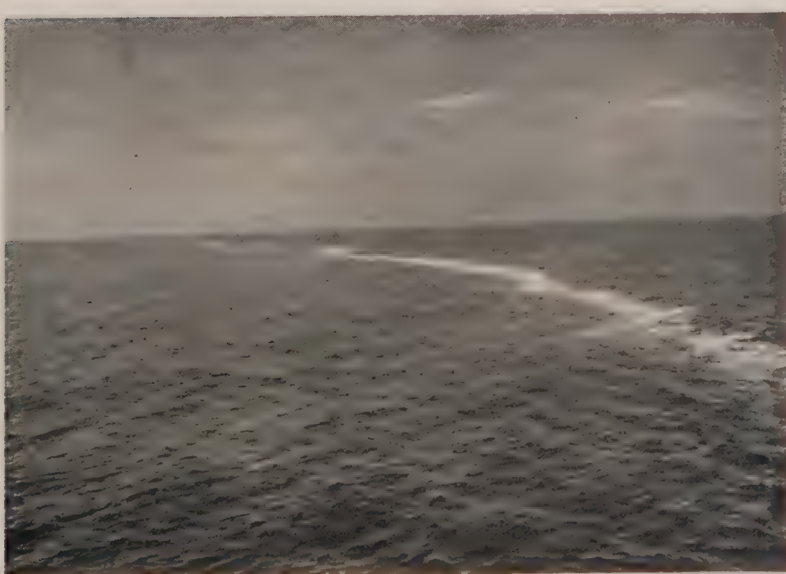


Fig. 9.



Fig. 10.

Figs. 9-10. These photographs were taken from a ship to show the current rip caused in Ishikari Bay when the wind blows from the south.

3 潮境の形態

前述の如く潮目の分布状態は，風向によつて著しく左右されているので，その形態をしらべるため，南風系の時を選び，1958年7～8月および，1959年7～8月にわたつて海洋観測を行つた。Fig. 11 は1959年度の観測点で，矢印およびその番号は，幼稚魚採集のための曳網個所である。

Fig. 12～17は，表面，2.5m，5m，10m，海底の塩分および水中照度（透過光量の百分率）を示し，Fig. 18～21は，st. 1～8，st.10～16，st.18～24，st.25～31における塩分，および照度の垂直断面図であり，Fig. 22，23 は河口より沖合の観測点を結んだ水温，水中照度および塩分の垂直図である。

潮目は，st. 5，6，12，13，14，20，21，22，27，28，33，34 附近を結ぶ線上に分布しており，その場所は風向によつて幾分移動する。Fig. 12，18，19，20，21 の斜線の部分が潮目で，この部分において河口より流出した河水と，湾流の海水が収斂しており，塩分の水平分布においても Fig. 12 でわかる如く潮目を境にして急激に変化しており，収斂の状態をはつきりあらわしている。

垂直分布においては，河水が湾内に流入して沖合に拡散する場合，海水とくらべ非常に低密度のため，垂直安定度がよく，なかなか混合がおこなわれにくく，皮流の状態となつて沖合に拡散している。すなわち河水と思われる巾は，水平分布において，st. 1～8 間では，st. 5 と 6 の中間から 8 の間であり，st.10～16間では，st.12から16迄，st.18～24間では，st.20から23と24の中間迄，st.25～31間では，st.27と28の中間より，st.30と31の中間迄というように次第に拡がつているが，垂直分布においては，河口附近では，水深が浅いせいもあるが，河水はほとんど海底まで影響しているが，st.15附込より沖合になると，2～4 mを躍層として上層に河水があり，それ以下は海水でなかなか混合がおこなわれないようで，しかも沖合に拡散するにつれて，河水と思われる層はますます薄くなつている。

照度の変化に対しては，石狩川そのものが，相当多量の有機，無機の懸濁場を有して，河水は泥濁色を呈

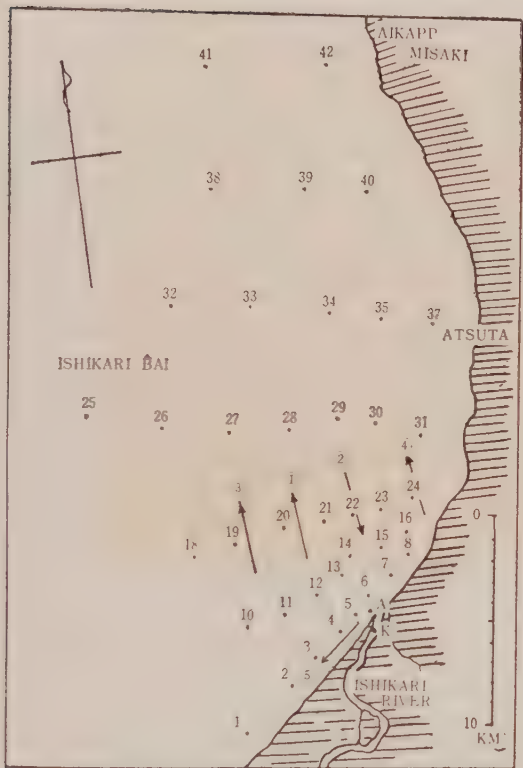


Fig. 11. Location of the hydrographic stations and towing of the laval-net operated by the "Ōmi Maru" in July and August 1959

しているため，河水のおよぶ範囲においては，海水にくらべて著しい光の吸収，散乱がおこなわれているので，潮目を境界にして塩分と同様照度は勿論，水色も著しい変化をしており，透過光量の百分率においては，河水のおよぶ範囲でその減衰が著しい。

石狩川河水によつて搬入される有機懸濁物が多いということは前述のとおりであるが，有機懸濁物の量は，過マンガン酸加里消費量によつて，その量を表わすことができるが；これについては海藻化学教室の小黒美樹氏より発表があるはずであるがこれを要約すると（筆者等はこれを酸素消費量で求めた），河水のおよんでいる海区は，沖合の海区とくらべて圧倒的にその量が多く，河口附近一帯が表面において酸素消費量が，7.72～22.75 mg/l の高い値を示しており，潮目附近では，河水側で 3.52 ～ 6.69mg/l の値が，海水側では，2.29～3.13mg/l と減少している。しかしその拡散状態は，塩分の場合と異り，沖合に行くに従つて，表面より中層，下層へとその分布の最高値が移動しておる。すなわちこれは海水中の有機懸濁物が，海中に溶在する強電解質の塩によつて凝集を起し，ゲル（Gel）状となつて次第に沈降してゆくものと思われる[8]。

次にこれらの有機物は海底に沈澱するが，それが潮目といかなる関係にあるかしらべる目的で，採泥により灼熱減量をおこなつて有機物の沈澱状態を調べたところ，Fig.24の如くなる。これによると，st. A. 6，13，21，28，33 という河口より沖合に向つて，ほぼ直線的な線上において，5.58%，12.02%，14.64%，18.16%，6.53%，8.74%という高分布を示すものと，st. 10，11，12，13，15，24. 31とほぼ沿岸に平行な線上において，5.04%，7.92%，11.43%，14.64%，13.77%，7.83%，8.48%という高分布を示す二通りの高分布地帯がみられる。これは丁度Fig. 2に示した潮目分布の見取図の1～4と5～7を重ね合せたものにほかならない。これからみると，沈澱は潮目の線に沿つて行なわれているものと思われる。潮目分布は風向によつて左右されているので，当然1年を通じて南風，北風とおこるその頻度により沈澱量が違つてくることになる。

過去3カ年におけるこの地の毎日（1日2度観測）の風向を石狩川治水事務所石狩事業所の観測日誌によると，

Table 1. Average direction of wind month by month at "Town of Ishikari" from 1957 to 1959

Wind Direction \ Month	1	2	3	4	5	6	7	8	9	10	11	12	Average
South	15.5	14.5	14.3	16.0	15.5	17.0	19.5	19.2	16.8	17.2	12.2	11.5	15.8
North	12.8	10.0	13.7	12.8	14.5	12.3	10.7	10.3	10.5	10.5	13.2	15.2	12.2
West	1.7	3.2	3.0	0.7	0.3	0.5	0.5	0	1.8	3.0	4.3	3.2	1.9
East	1.0	0.3	0	0.5	0.7	0.2	0.3	1.5	0.8	0.3	0.3	1.7	0.6

※ 風向のSouthは，ＳＥ/Ｅ～Ｓ～ＳＷ/Ｗは総てSouthとし，ＮＷ/Ｗ～Ｎ～ＮＥ/ＥはNorth，Westは，ＷＮＷ～Ｗ～ＷＳＷ，Eastは，ＥＮＥ～Ｅ～ＥＳＥとして計算した。

Table 1 の如くで，南風が多く次に北風となつており，河口より沖合にほぼ直線的にできる潮目の回数が多いこと，その線上に沈澱量の多いことが一致している。

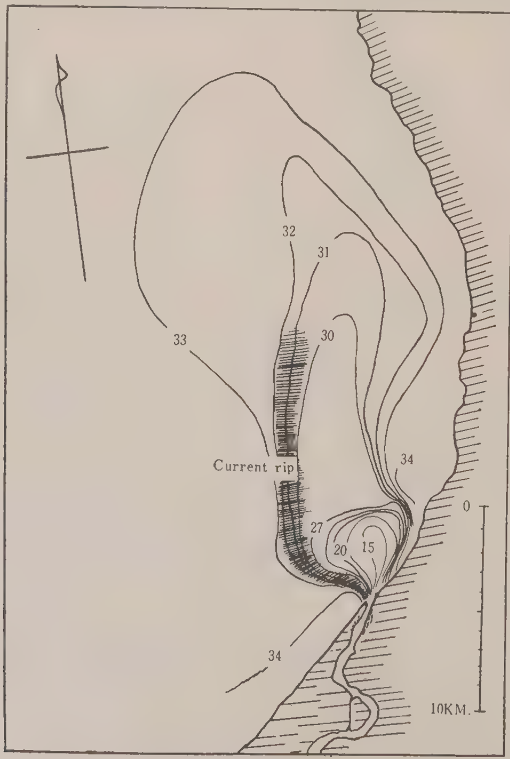


Fig. 12. Horizontal distribution of salinity of the surface water

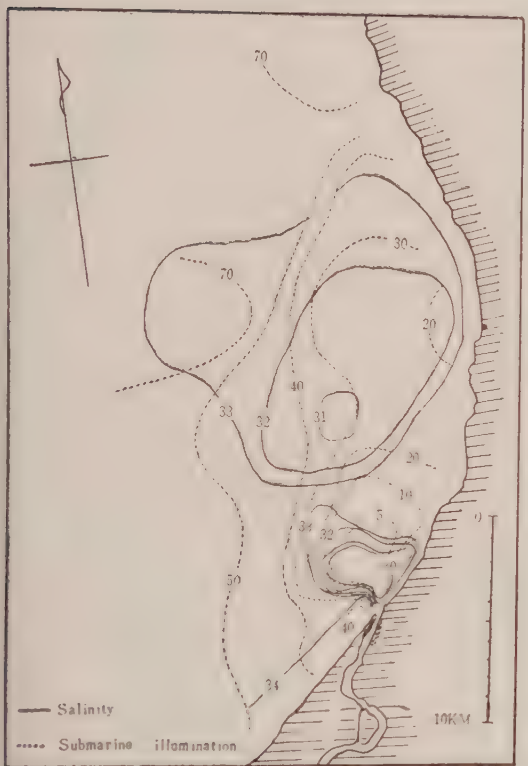


Fig. 13. Horizontal distribution of salinity and submarine illumination at 2.5m depth from the water surface

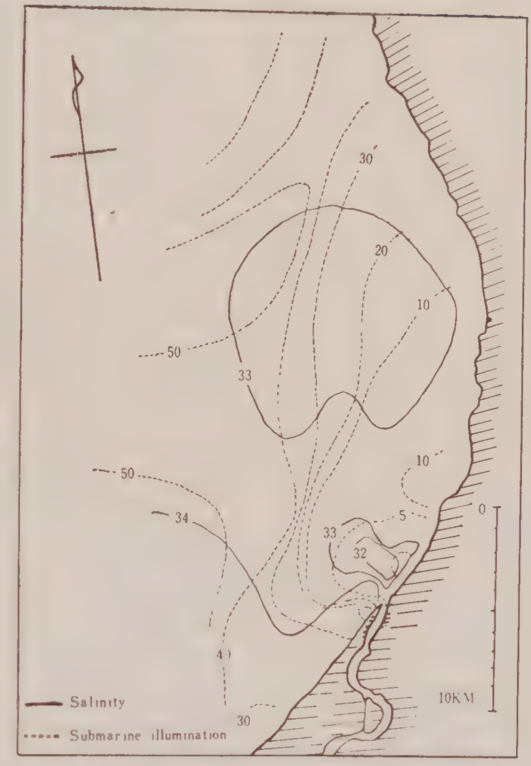


Fig. 14. Horizontal distribution of salinity and submarine illumination at 5m depth from the water surface

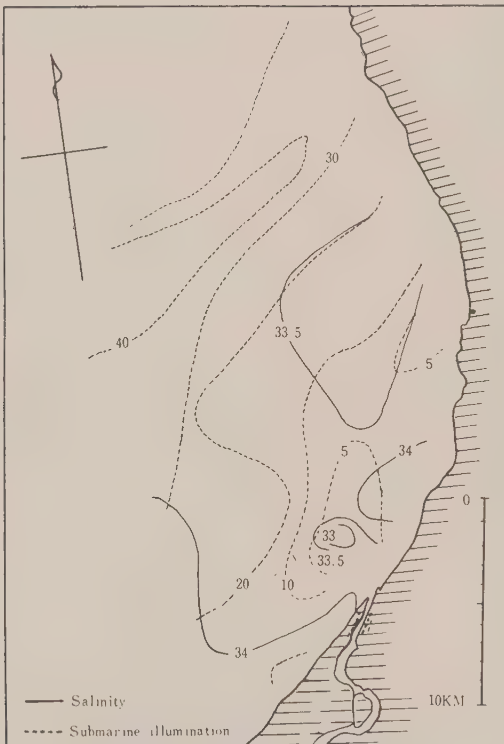


Fig. 15. Horizontal distribution of salinity and submarine illumination at 10m depth from the water surface

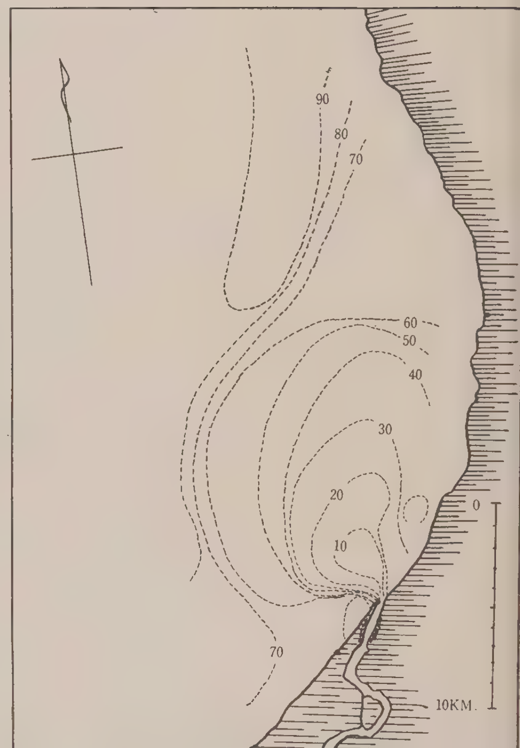


Fig. 16. Horizontal distribution of submarine illumination at the surface



Fig. 17. Horizontal distribution of salinity
at the sea bottom

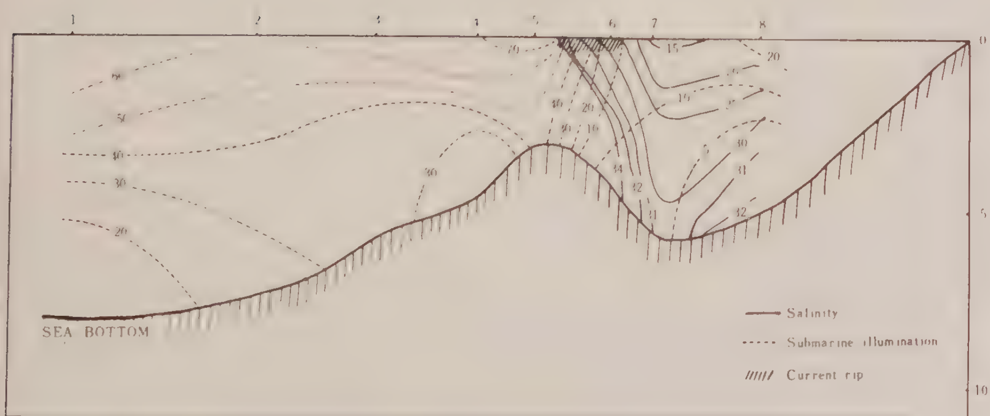


Fig. 18. Vertical distribution of salinity and submarine illumination
from st. 1 to st. 8 in Ishikari Bay

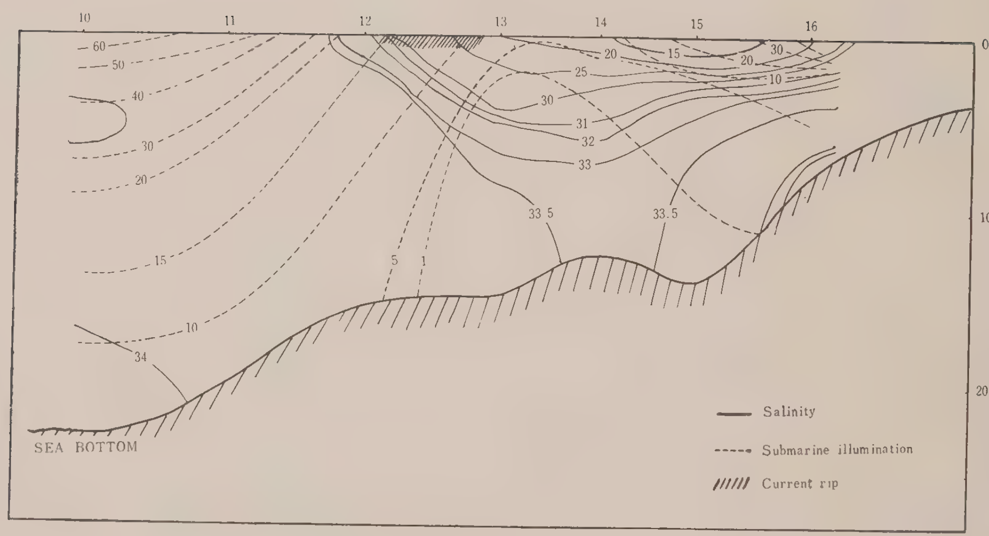


Fig. 19. Vertical distribution of salinity and submarine illumination from st. 10 to st. 16 in Ishikari Bay

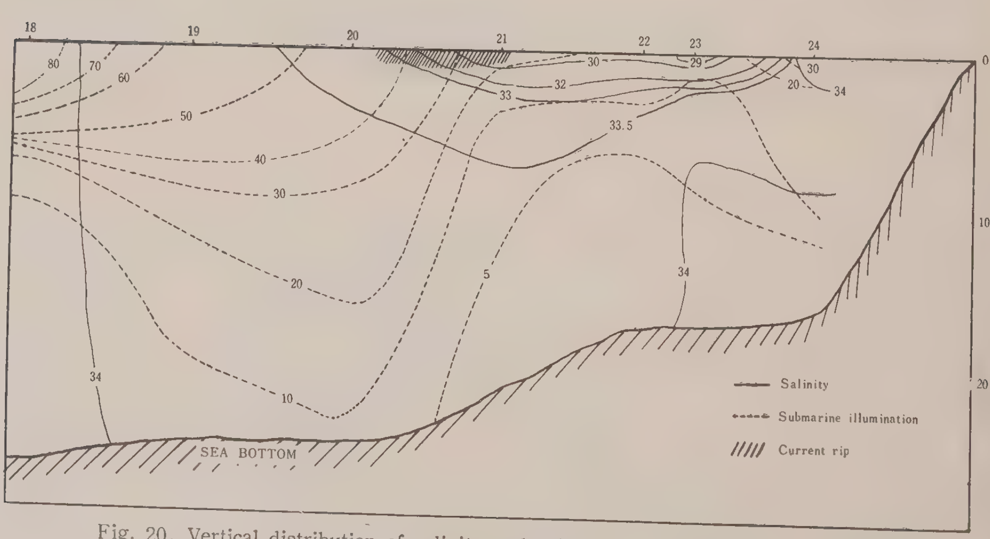


Fig. 20. Vertical distribution of salinity and submarine illumination from st. 18 to st. 24 in Ishikari Bay



Fig. 21. Vertical distribution of salinity and submarine illumination from st. 25 to st. 31 in Ishikari Bay

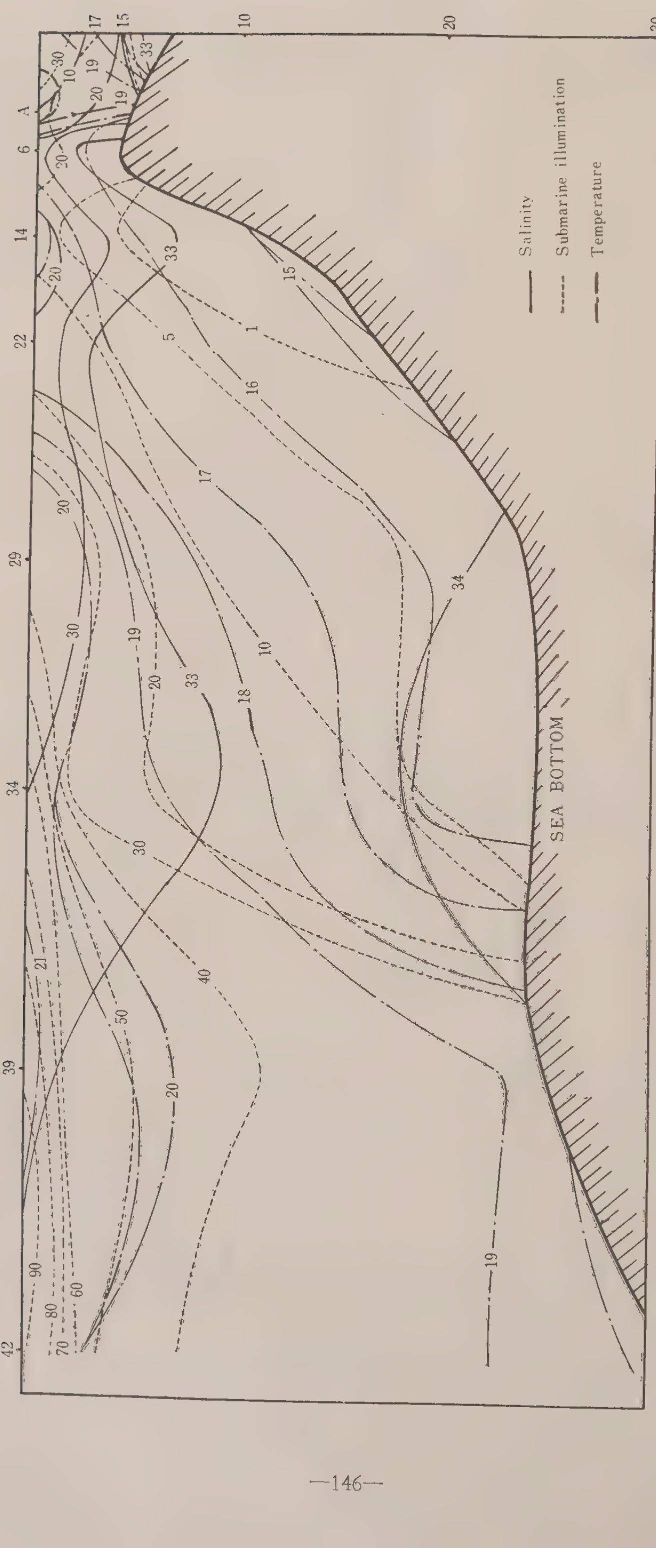


Fig. 22. Vertical distribution of temperature, salinity and submarine illumination from st. K to st. 42 in Ishikari Bay

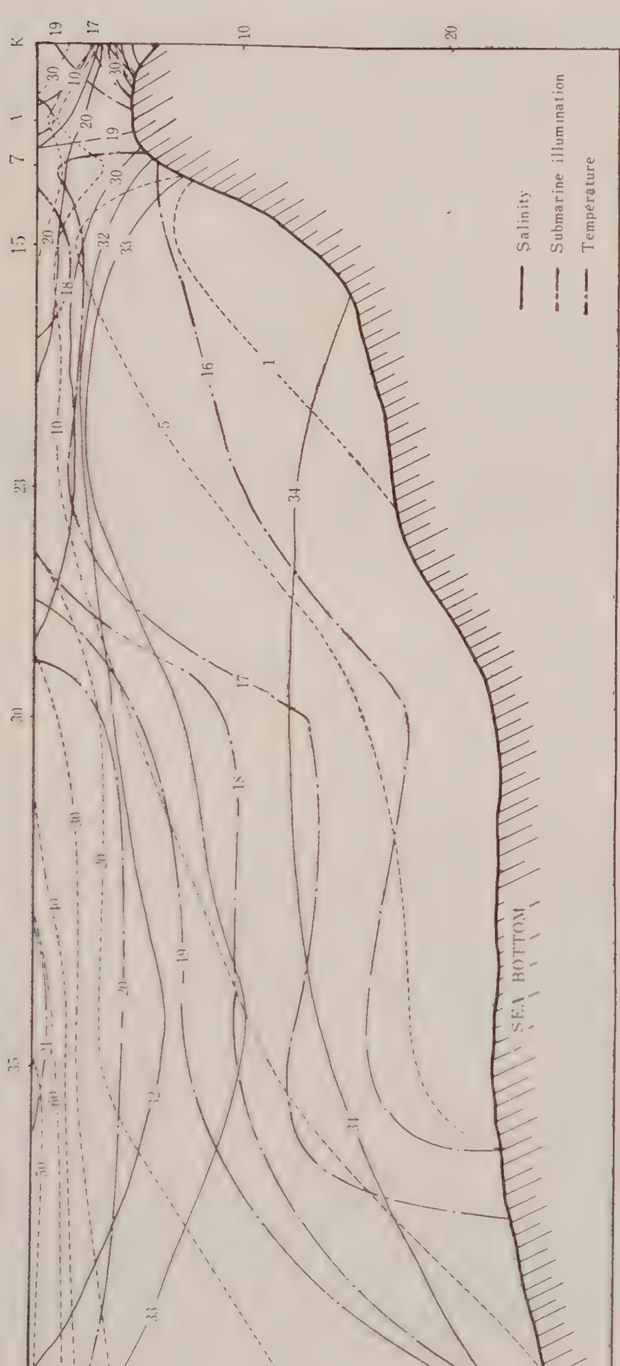


Fig. 23. Vertical distribution of temperature, salinity and submarine illumination from st. K to st. 40 in Ishikari Bay

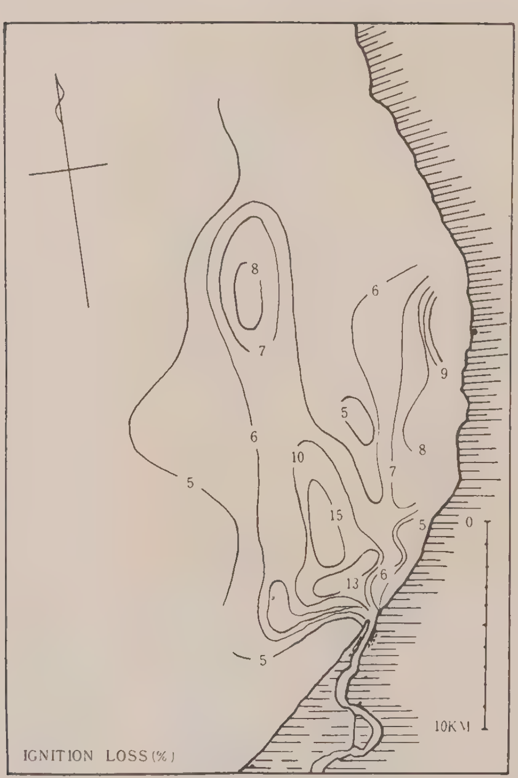


Fig. 24. Horizontal distribution to show the ignition loss in Ishikari Bay

4 潮境附近の反射について

ここで考えられることは，潮目の部分における反射と，河水が皮流となつて流出しているため，その皮流と海水との境界面における塩分差，温度差による反射と，プランクトンおよび，その他の水中懸濁物による反射が考えられるので，株式会社産研のＮＳ—300Ａ型，周波数200kc,発射回数，浅150R/mの魚群探知機を用い，これに抵抗減衰器を併用して，標準置換体として３寸径のガラス玉を使用して，間接測定法によりそれぞれの反射損失を測定した。

（４）—ａ　潮目部分の反射

潮目は両水塊の衝合線であるので，その附近の海水の動きは複雑であり，また塩分温度の不連続，海水の衝合等により多数の泡を発生しているので，潮目通過の節はかなり強い反射が得られた。しかし Fig. 18の st. 5 〜 6 間の潮目は図でもわかるように潮目そのものの垂直傾度が急なため，航行中瞬間的にその上を通過してしまい，ほとんど記録を得ることはできなかつたが， Fig. 19，20の潮目においては表面より５ｍまでに56.5 〜 74.5db の反射損失が求められた。この反射記録でもわかるように表面より５ｍまで連続したものであるところより，塩分差というより含有気泡による反射と考えた方が適当と思われる。

Fig. 25, 26 は st.12〜13間および st. 20〜21間の潮目上を蛇行した記録であり， 前述のように０〜５ｍまでに海水の攪乱と，それによつて生ずる気泡層と思われる反射が存在している。

（４）—ｂ　塩分および温度の変化（現場密度の変化）による反射

塩分変化，温度変化が急な場合反射が行なわれるが，その基本的考え方については前号[1]で述べた通りであり，またそれ等が個々に存在する場合の水槽内における実験値については橋本[6]の発表があり確認されている。しかし河水と海水の衝合しているような場所においては，これらが個々に独立して存在することはほとんどなく，　分変化の顕著なところは温度変化も顕著であり，温度変化の存在する個所は塩分変化も存在するので，超　波の反射についてはこれらを個々に論ずることはできないので，筆者等はこれを数値的に現場密度の変化として取扱つた。

その最も顕著な例として河口附近は，Fig. 22，23 でわかる如く，遡上海水が相当上流まで河底を遡上しており，この上を河水が流出しているので，この面において塩分,水温の不連続が生じており,これに相当する個所より反射記録が得られ，その反射損失を実測したところ，39〜49dbの非常に強い反射値を得た。Fig. 27および Table 2は，st.Kにおける諸量であり，Fig. 28，29はその場所における反射記録である。

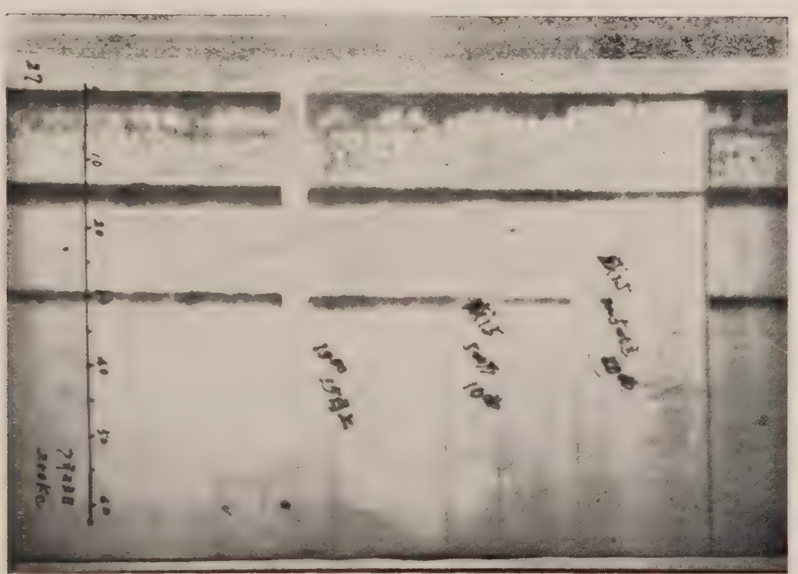


Fig. 25. Soner echo due to current rip



Fig. 26. Soner echo due to current rip

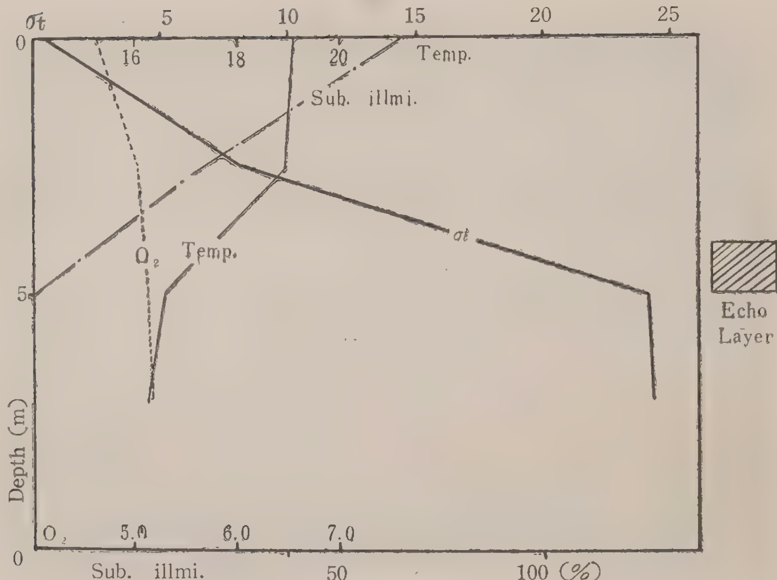


Fig. 27. Graph of vertical distribution of temperature, oxygen (O_2) density in situ (σt) and submarine illumination at st. K as shown in Fig. 9

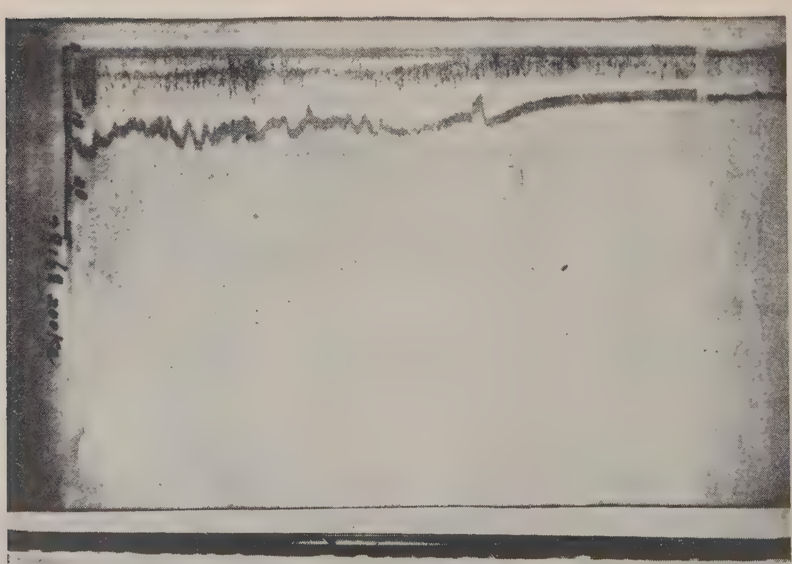


Fig. 28. Sonar echo due to difference of density in situ (σt) obtained at the estuary of the Ishikari River

Table 2. Oceanographic data observed at st.K as shown in Fig. 9

Depth (m)	W.T. (°C)	Cl (‰)	S (‰)	σ_t (g/L)	O_2 (cc/L)	Sub. illumi. (%)
0	19.1	0.831	1.529	0.58	4.59	71.42
2.5	18.9	6.590	11.925	8.30	5.03	30.71
5	16.5	18.269	33.005	24.12	5.14	0
7	16.3	18.300	33.062	24.31	5.14	0

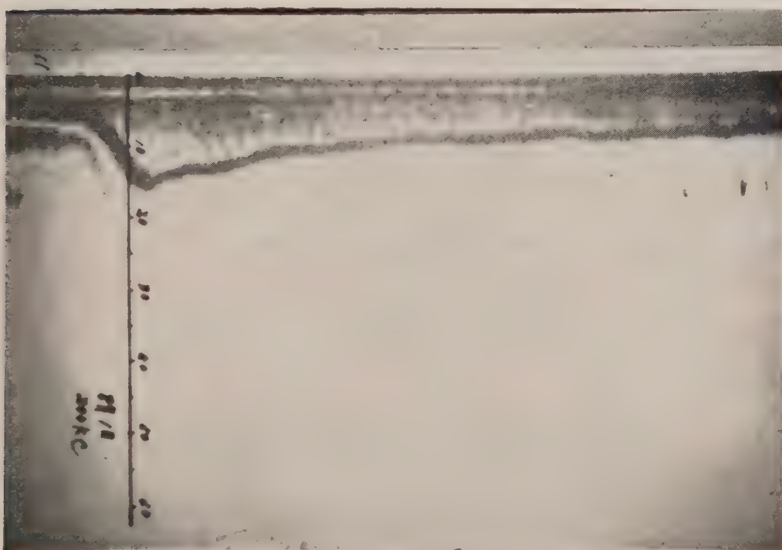


Fig. 29. Sonar echo due to difference of density in situ (σ_t) obtained at st. 13 as shown in Fig. 9

Table 2 でわかる如く，水温は5m 層までに0.9°C/mの変化をし，塩分は6.295‰/mの変化をしており，これを σ_t に換算すると，4.4gr/l/mの変化となり，4.5～5m 以下は完全な海水となつていて，塩分，水温もほとんど変化がないことより，同層よりの反射に間違いなく，これに加うるに上層は 1.8m/sec という河水の流動があり，下層は流速ほとんど零という海水のため，流れの不連続も加わつて，かように大きな反射が得られるものと思われる。

石狩川の遡上海水の状態については，福島[7]は，供水型，減水型，およびその中間型があると述べているが，今後は200kc 音響探知機を用いることにより，反射層がどこにあるかを知れば，ある程度遡上海水の水位の状態を知ることができると思う。

次に沖合の状態であるが，Fig. 22，23 でわかる如く，河水は2～4m を躍層として皮流となつて沖合に拡散しているので，音響探知機の送受波器が水中 1.5m 入つているところから，ごく河口近くの躍層が比較的深い場所以外は，発振線と反射の記録が一致してなかなかよい記録を得ることはできなかつたが，53.5～74.5db（主に 60db 台）の反射損失を実測し得た。Fig. 30，31 はその時の記録を示し，Table 3，4 はその場所の諸量であり，Fig. 32，33 はその垂直図である。



Fig. 30. Sonar echo due to difference of density in situ (σ_t) obtained at st. 13 as shown in Fig. 9

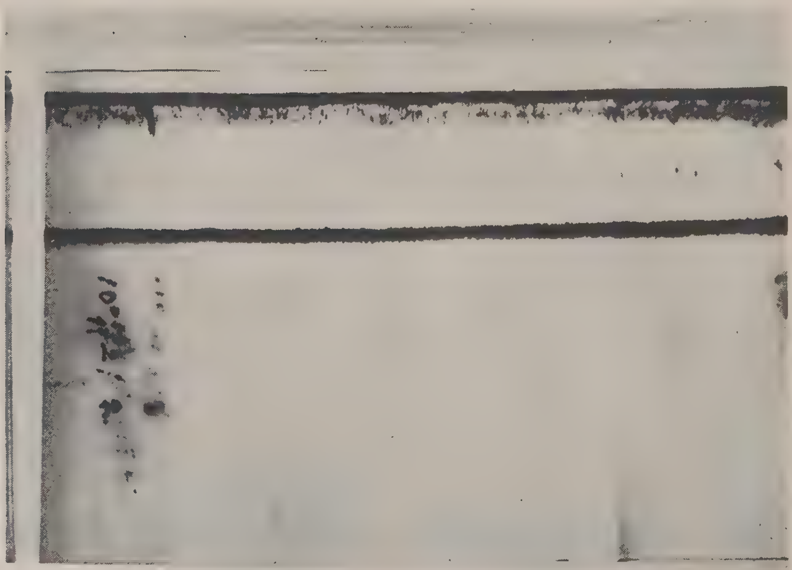


Fig. 31. Sonar echo due to difference of density in situ (σ_t) obtained at st. 21 as shown in Fig. 9

Table 3. Oceanographic data observed at st. 13 as shown in Fig. 9

Depth (m)	W.T. (°C)	Cl (‰)	σ_t (g/L)	O_2 (cc/L)	Sub. illumi. (%)
0	17.7	11.313	14.84	5.11	9.14
2.5	16.4	14.966	19.89	5.42	0.32
5	15.7	17.784	23.63	4.57	0.22
7.5	15.2	18.406	24.60	5.38	0.11
10	15.2	18.743	25.07	5.64	0
15	15.0	18.730	25.10	5.68	0

Table 4. Oceanographic data observed at st. 21 as shown in Fig. 9

Depth (m)	W.T. (°C)	Cl (‰)	σ_t (g/L)	O_2 (cc/L)	Sub. illumi. (%)
0	18.1	15.509	19.95	5.50	22.45
2.5	17.2	18.254	23.93	5.72	15.53
5	17.0	18.396	24.19	5.80	8.33
7.5	17.0	18.489	24.28	5.51	5.68
10	16.9	18.689	24.62	5.60	5.30
15	16.8	18.795	24.76	5.65	4.32
19	15.2	18.800	25.15	5.91	3.18

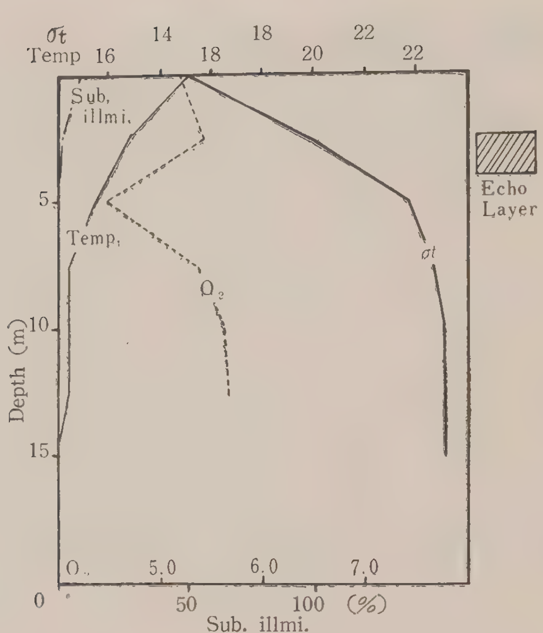


Fig. 32. Graph of vertical distribution of temperature, density in situ (σt), oxygen (O_2) and submarine illumination at st. 13 as shown in Fig. 9

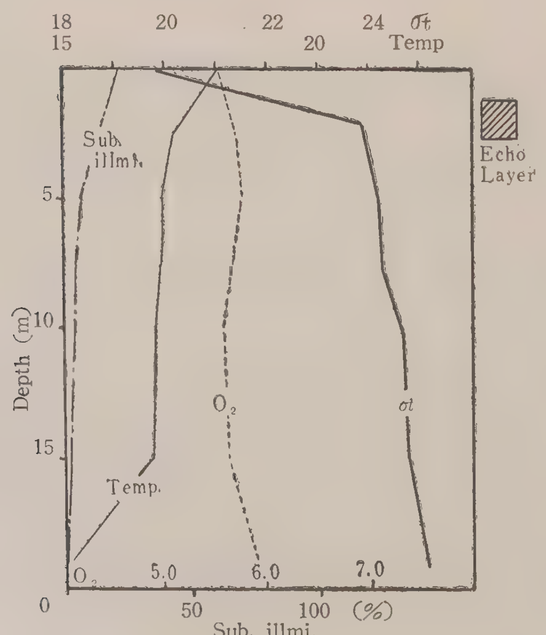


Fig. 33. Graph of vertical distribution of temperature, density in situ (σt), oxygen (O_2) and submarine illumination at st. 21 as shown in Fig. 9

これを河口を含め海区全体について水深1m当りのσtの変化量について Plot すると，Fig.34の如くなる。

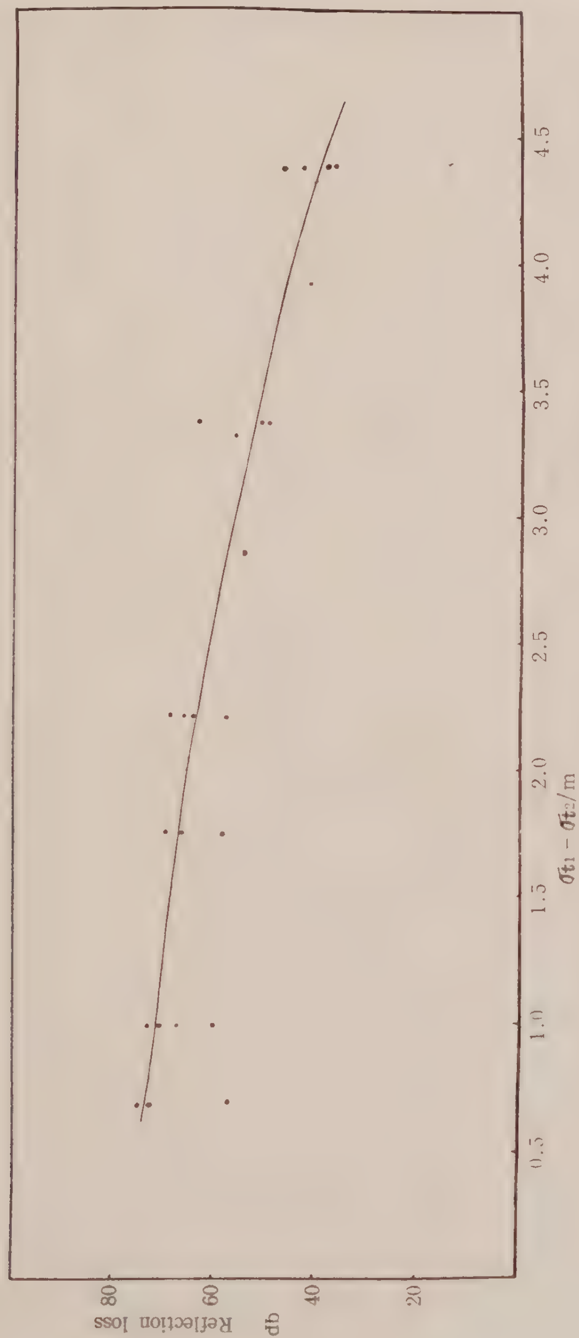


Fig. 34. Reflection loss due to difference of density in situ (σt)

(4)―C　懸濁物質の反射について

波長に比べ極く小さい懸垂物の反射損失は，理論的にはすでに述べた如くである。[1] 一般海洋上であると，懸垂物というと主にプランクトン群を指しているが，前述のようにこの海区は河川からおびただしい浮泥類が流出しているので，それらが沈澱して行く過程において海中に懸濁状態にあり，それによる反射と考えられる点が非常に多かつたので，筆者等はプランクトン採集（口径30cm，側長95cmの閉鎖ネット，国産篩絹XX13製使用）により，各層の垂直分布を測定した（北海道区水産研究所飯塚篤氏に依頼），しかし垂直分布においては，D.S.L. 解明のために大方の人が色々実験されているそれらの諸量よりはるかに小量な，0.1〜0.8cc/m という分布量で，これのみによる反射層とは当然考えられず，しかも反射海域が潮目より外洋水側に出るとほとんどかような反射も出ず，Fig. 24 の沈澱の模様と思いあわせて，やはり有機懸濁物等による反射と断ぜざるを得ない，ただこの懸垂物の実態については，今回は明らかにすることができなかつた。

海中に懸濁物がある場合，透過光線はそれによつて散乱をおこなう，海面上の照度が I_0 である場合，鉛直消散係数（Extinction coefficient）κは，（I_Z：深さ Z で測つた照度）

$$K=2.30\ (\mathrm{Log}\ I_0-\mathrm{Log}\ I_z/z) \quad (1)$$

で表わされるので[3] 各層における κ を求めれば，κ の価が大きい層においては，散乱物質が多いということになるので，κ と反射層における反射損失の関係をとつたところ，Fig. 35 の如くなり，Fig. 36 はκ の値が 15〜17m層において 0.20から0.70 に変化した層に反射のある st. 23 の反射記録であり，Table 5は，その地点における諸量で Fig. 37 はその垂直図である。勿論外洋において，これと等しいKの変化がある場合，これだけの反射損失が得られることには疑問はあるが，少くともKの変化により，反射損失の値が変化するということは，κ に関係する物質によつて反射がなされていることは確かであるので，今後この問題について定量的に研究を重ねて行く予定である。

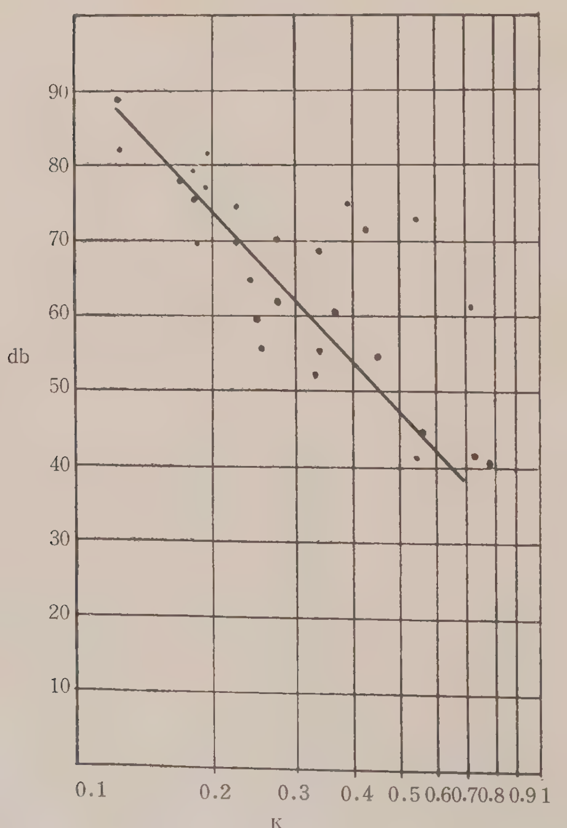


Fig. 35. Reflection loss due to the variation of extinction coefficient

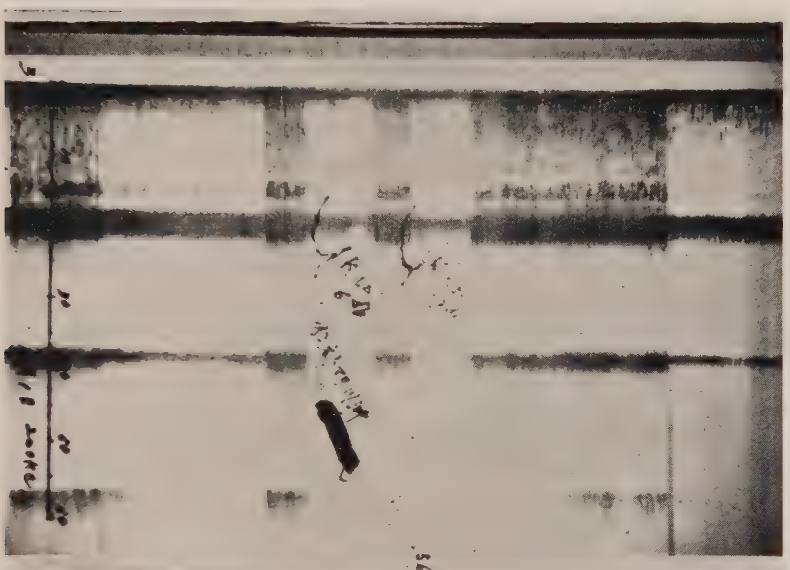


Fig. 36. Sonar echo due to suspended matters obtained at st. 23 as shown in Fig. 9

Table 5. Oceanographic data observed at st. 23 as shown in Fig. 9

Depth (m)	W.T. (°C)	Cl (‰)	σ_t (g/L)	O_2 (cc/L)	Sub. illumi. (%)	Extin. coeff. (κ)
0	17.7	16.022	20.75	5.96	15.59	18.58
2.5	16.6	18.613	24.54	5.66	6.77	0.35
5	16.4	18.768	24.84	5.49	5.81	0.06
7.5	16.3	18.840	24.95	5.41	5.27	0.04
10	15.9	18.835	25.04	5.53	3.29	0.16
15	15.1	18.842	25.23	5.67	1.29	0.20
17	14.9	18.320	25.25	5.75	0.32	0.70

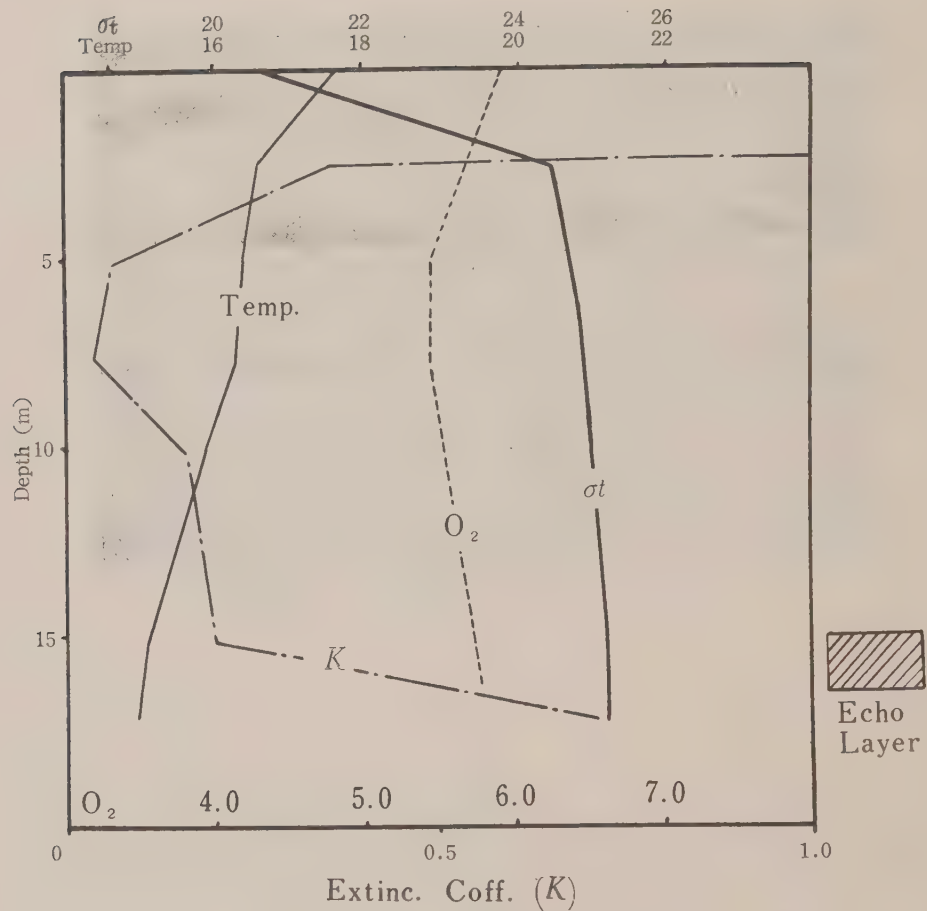


Fig. 37. Vertical distribution of temperature, density in situ (σt), oxigen (O_2) and extinction coefficient

5 潮目附近の幼稚分布について

潮境に魚族が密集することは，古来からの漁場を一見すればすぐわかることであるが，このような河水の流出によつてできている潮境附近はいかなるものであるかを知ることも重要なことと考え，三枚網により，表層，中層，底層と各層の漁獲試験をおこなつたが，試験回数の不足により確固たるデータを作成することができなかつた。幼稚魚の分布については，Fig. 9で示したように5カ所において，それぞれ30分間にわたつて，口径 130cm，側長 450cm の㊉型稚魚ネットにより表層曳をおこなつた。①は潮目上（あわの上）②は潮目より河水側，③は潮目より海側，④は比較的沿岸に近い所，⑤は沿岸である。Table 6 はその時採集された幼稚魚の分類表であり，Table 7 は曳網個所附近における諸データである。これによると，潮目上の分布が淡水性，鹹水性を問わず圧倒的に多い。その原因については，水塊の動きよりみて集るというより，両性のものが寄せられたと考えた方が適当と思われる。②は河水の影響の非常に強いところであるが，淡水性のみならず相当数の鹹水性のものがみられ，③については，河水の影響のほとんどない個所である。これらについては，水中照度の強弱によつて表層の分布に差が生じたものと思われる。すなわち稚魚その他の生

物分布は，夜間は表層に上昇し，日中は深くもぐるという垂直移動をおこなつているといわれるが，非常に濁りの強い河水側では，表層において透過光量が著しく減衰されてしまうので，濁りの少い海水側とくらべると，Table 7 に示す如く，その水中照度が著しい差が生じるので，それが原因して③で全然採集されないという結果が生じたものと思われる。なお③における 15m 層の水中照度が 18200Lux であつたので，①，②，④における 2.5m 層とが大体同じ水中照度であつたということになる。

以上採集された幼稚魚の数のみについて考察したが，漁場的にみて，どうかという問題については，やはり採集回数の不足より確言はできないが，少くとも潮目上において，カタクチイワシ，サンマ，サヨリ，マサバ，クロゾイ，メバル等有用種の幼稚魚が多数採集されたということは，今後の石狩湾の漁場構成上非常に有効なことと考える。なお採集は分布上に時期的差のないよう同一日の午前10時より午後3時までになされたものである。幼稚魚の鑑定は，本学動物学教室の小林喜雄氏によるもので，それらの組成，特徴等については，いずれ同氏より専門的立場にたつて発表されることになつている。

Table 6. Indicating the catch of larval and youngs of various species of fish around the current rip in Ishikari Bay caused by the River Ishikari running into that bay (length mm)

specific name of fish	Number of catch (length)				
	1	2	3	4	5
Katakcuhiiwashi	116(5.0-11.0)	469(3.5-21.5)		139(9.0-21.5)	11(6.5-14.0)
Sanma	9(5.0)				
Sayori	21(5.0-52.0)	5(29.4-70.0)			
Itoyo	773(14.8-30.0)	119(18.5-28.0)			
Youjiuo	oyauo 3(188-195) 7(7.0-30.0)	1(28.0)			
Kitanoumiuma	1				
Boraka no isshu	6(9.3-19.1)				
Masaba	100(3.0-25.5)	20(3.0-11.0)			
Mejina	1(20.2)				
Himehaze	oyauo 1(89.0)				
Kawahagi	4(4.5-7.5)				
Torafugu no isshu	1(12.1)				
Ezomebaru	39(17.0-32.4)				
Murazoi	7(21.0-25.6)				
Mebaru no isshu	73(13.0-25.6)	1(16.4)			
Mebaru no isshu	52(15.7-30.7)				
Hirame	1(11.0)			3(8.0-9.0)	
Gyoshu fumei A	3(14.5-16.3)				
Gyoshu fumei B	1(2.5)				
Sanma ran	large number	small number			
Kurage			large number	small number	large number

Table 7. Oceanographic data observed at five fishing grounds

	depth (m) \ st.	1 (st.21)	2 (st.22)	3 (st.19)	4 (st.24)	5 (st. 5)
Submarine illumination (Lux)	surface	125,000	115,000	120,000	100,000	95,000
	0.1	28,000	20,000	70,500	33,000	70,000
	2.5	20,500	12,500	70,500	18,700	
	3.0	—	—	—	—	43,000
	5.0	10,400	11,000	58,600	17,500	
	10	6,600	2,500	26,600	7,100	
Salinity (‰)	0.0	28.024	29.785	33.666	34.121	34.239
	2.5	32.978	33.468	33.836	33.926	
	3.0	—	—	—	—	34.108
	5.0	33.235	33.982	33.860	33.951	
	10	33.764	33.849	33.912	34.054	
Temp. (°C)	0.0	18.1	17.6	18.6	16.5	16.7
	2.5	17.2	17.1	17.5	16.4	
	3.0	—	—	—	—	16.5
	5.0	17.0	16.7	17.2	16.2	
	10	16.9	15.4	16.7	15.6	

6 考察並びに結論

以上石狩川河水の流出によつてできる潮境の形態，その附近の反射，および幼稚魚の分布について述べたが，専門以外のことも多く，資料の採集等に不手際もあり，完全なものはできなかつたが，これを要約すると

(1) 潮目分布については，南風型と北風型の2つに大別でき，前者は河口より沖合にほぼ一直線に分布し後者は，沿岸にほぼ平行にできる。

(2) 南風型における河水の沖合への拡散状態は，低密度のため，皮流の状態となつて沖合に流去し，北風型の場合は，沿岸に堆積される。

(3) 河水のはこぶ懸濁物の沈澱は，南風型，北風型を問わず，潮目の線にそして多量に沈澱するようである。

(4) 潮目部分の反射については，水塊の衝合により生ずる乱流と，それによつて生ずる気泡層よりの反射と思われる反射があり，その反射損失は 60db 前後であつた。

(5) 塩分差，温度差による反射については，現場密度（σ_t）が 1 m 当り約 4.0$^{g/l}$/mの変化がある場合，44〜5db 約 0.7$^{g/l}$/m の変化の時が約 70db 前後の反射損失であつた。

(6) 懸垂物の反射については，プランクトン群というより，有機懸濁物の層と思われるものよりの反射があり，消散係数にして，約 0.55 で 45db 前後，約 0.18 で 80db 前後の反射損失を得た。

(7) 幼稚魚の分布については，潮目の部分にて多数採集されたが，これは寄せられたものと思われる，その他の地点の分布については，水中照度が影響しているものと思われる。

7 謝　　辞

本研究は，専門外の項目も多く，幼稚魚の鑑定は，本学部動物学教室の小林喜雄氏，過マンガン酸加里消費量，灼熱減量の測定には，海藻化学教室の小黒美樹氏，プランクトンの定量および種別判定には，1959年度は北水研の飯塚篤氏，1958年度は本学部淡水養殖教室の富士昭氏をわずらわしたもので，深く謝意を表する。実験に当つては，開発庁石狩川治水事務所，石狩支庁水産課，石狩水産振興会，石狩漁業協同組合，また飛行機搭乗の便宜を承諾された北部方面航空隊等の関係各位に対し厚く謝意を表する。また直接実験に際し手伝つていただいた本学学生，加納隼人，佐野崇，内田茂，桃井修一，谷口滋穂，および本教室研究補助員，佐藤輝彦君に対して併せて謝意を表する。

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CHEMICAL STUDIES ON THE MEAT OF "SUKETODARA" (*THERAGRA CHALCOGRAMMA*)-I

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INTRODUCTION

Theragra chalcogramma (PALLAS) is called "Suketodara" or "Sukesodara" in Japan. In Korea it is called "Mentai". It is called "Alaska pollack" in English.

This is a useful fish belonging to the Gadidae as do cod also. Typical genera of the Gadidae are *Gadus* and *Theragra*.

Theragra chalcogramma is distinguished from the ordinary cod (*Gadus macrocephalus*) by having more slender body, the lower jaw is longer than the upper, the barbel on the chin is much smaller than on the cod and the caudal fin is more or less emaciated posteriorly. The body height is about 1/6 of the length; the snout is sharply pointed; in the large mouth the maxilaries extend to below the middle of the eye; the large eye is placed on the head of about 4 inches.



Fig. 1. "Suketodara" (*Theragra chalcogramma*)

The anus opens below between the first and second dorsal fins. The ventral fins are filamentous distally and their tips reach to the anus. The body color is brownish in the back part, white in belly part; the two interrupted horizontal dark bars on the side are irregularly bordered. The total average length reaches to about 600 mm.

The "Suketodara" occurs in the waters of Japan Sea from Yamaguchi Prefecture northward to the Bering Sea and off Korea; it is scarce on the Pacific coast of Japan. Differing from cod (*Gadus macrocephalus*) the "Suketodara" lives in the middle layers of waters at about 200 m depth; in the Bering Sea they swim near the surface of the water; very frequently they are devoured by the fur-seals.

The spawning season of "Suketodara" extends from December to February in the waters off Korea, and February to April in waters near Hokkaido and elsewhere. The separate, pelagic eggs are spherical and each measures 1.35 to 1.45 mm in diameter; the egg membrane is smooth, the yolk has no cleavage and the oil-globule is absent. The larvae just before hatching are colored by only black chromatophores which are uniformly scattered over the body. The fishing season of this fish lasts from December to February, and the fishing gears include set-line, gill-net and small-trawl. The fish is utilized as cod is, and the salted eggs ("Momijiko") of this fish are especially appreciated; in Korea this fish, being one of the important aquatic products, is dried after being frozen.

The catching of this fish totaled 280 thousand tons in 1958, with 90% of them being caught in Hokkaido.

The skeletal meat is utilized as fresh raw fish (for human consumption), eggs are salted and the liver is utilized as raw material for liver oil in Hokkaido.

With increase in the demand for fish pastes (e.g. "Kamaboko" or fish sausage and fish ham), requiring a great amount of raw material, the use of "Suketodara" is being developed with keen interest as a raw material for such products.

A small amount of the catch has been utilized as raw material of fish pastes, but as the freshness of this fish meat falls easily, the elasticity of the paste products ("Ashi" in Japanese) is lost remarkably, therefore the use of this fish is not looked upon with favor. The loss of elasticity has been explained recently from the view point of protein chemistry. But there are many unsolved problems remaining today in respect to the chemistry of the use of this fish.

As described in this paper the present authors have tried to clarify some little known properties of this fish meat in order to utilize it.

They have tried to study the fish from the points of view of general chemical analysis, histology and protein chemistry.

I. GENERAL CHEMICAL ANALYSIS OF "SUKETODARA" MEAT

In order to know the chemical constituents of the "Suketodara" meat, the general chemical composition and distribution of nitrogens and kinds of amino acids were determined.

1. Seasonal variation in general chemical composition

In general, the chemical composition varies remarkably by seasons of spawning. The "Suketodara" disappears from sight for some seasons in Hokkaido. So the authors have estimated monthly the general chemical composition of "Suketodara" meat from October to March of the succeeding.

(1) Experimental method

Fresh "Suketodara" caught in waters near Hakodate, Hokkaido, was used as the sample. The fish was filleted, and the meat was removed from the skin. The meat was homogenized and used as sample. Items estimated were water content, protein, fat and ash.

(2) Results

The results obtained are shown in Table 1.

As seen in Table 1, the amount of water-content was maximum in December with decrease afterward, then showed the minimum in March of the next year in the present experimental period. The difference in the amount of water content between the maximum

Table 1. Seasonal variation of proximate components of "Suketodara" (*Theragra chalcogramma*) meat

Items / Date	Water content (%)	Crude fat (%)	Crude ash (%)	Pure-protein (N ×6.25) (%)	Non-protein -N (%)	Total-N (%)	Crude protein (%)
25/Oct. (1953)	81.18	0.68 (3.61)	1.09 (5.79)	14.13 (75.1)	0.09 (0.48)	2.35 (12.48)	14.69 (78.0)
5/Nov.	81.15	0.73 (3.87)	1.21 (6.42)	14.75 (78.3)	0.18 (0.96)	2.54 (13.46)	15.88 (84.3)
9/Dec.	82.11	0.70 (3.91)	1.11 (6.21)	13.38 (74.8)	0.35 (1.96)	2.49 (13.91)	15.56 (87.0)
18/Jan. (1954)	81.06	0.53 (2.80)	1.03 (5.44)	13.69 (72.2)	0.33 (1.74)	2.52 (13.30)	15.73 (83.1)
10/Feb.	80.71	0.84 (4.36)	1.18 (6.12)	15.06 (78.0)	0.24 (1.24)	2.65 (13.73)	16.55 (85.9)
16/Mar.	80.31	0.93 (4.72)	1.23 (6.25)	16.75 (85.1)	0.20 (1.01)	2.88 (14.61)	18.00 (91.5)

Note : Number in parenthesis shows the percentage of each component per dried matter

and the minimum was about 2 %.

The amount of crude fat in raw meat showed minimum in January, but increased remarkably from February to March. The increased amount was 0.5 ~ 0.97 %.

The amount of ash was larger in November and March than in other months.

The total amount of nitrogen was considerably small in December. The amount of protein-nitrogen increased or decreased proportionally with the total amount of nitrogen. The maximum amount of protein-nitrogen was shown in March and the minimum in December.

The spawning season of the "Suketodara" is considered to be from January to April. The samples which were used by the authors in February and March were completely matured before the spawning. Therefore, the amounts of protein, fat and ash in those seasons were large while the water-content was less than in samples in other months. From the results showing the percentage of each chemical component per dried matter, the same conclusion as that stated above was also reached.

2. Differences of chemical compositions according to various factors

In general, the chemical components of fish meat are different owing to various factors, e.g. age, sex, size, etc.

Here, the authors have estimated the amounts of the general components in the "Suketodara" meat which vary with those factors.

(1) **Experimental method**

The items estimated were the same as those described in the previous experiment.

(2) **Results**

Results obtained are shown in Tables 2 ~ 4.

Table 2. Difference of proximate components of "Suketodara" (*Theragra chalcogramma*) meat by age

Items / Kinds	Water content (%)	Crude fat (%)	Crude ash (%)	Total-N (%)	Pure protein-N (%)	Crude protein (%)	Pure protein (%)
Young	83.01	0.49 (2.88)	1.08 (6.36)	2.45 (14.42)	2.05 (12.08)	15.31 (90.2)	12.81 (75.4)
Adult	80.31	0.93 (4.72)	1.23 (6.24)	2.80 (14.22)	2.50 (12.70)	17.50 (88.9)	15.61 (79.3)

Note : Number in parenthesis shows the percentage of each component per dried matter

Table 3. Difference of proximate components of "Suketodara" (*Theragra chalcogramma*) meat by sex

Items / Sex	Water content (%)	Crude fat (%)	Crude ash (%)	Total-N (%)	Pure protein-N (%)	Crude protein (%)	Pure protein (%)
Male	81.71	0.64 (3.50)	1.03 (5.64)	2.49 (13.61)	2.14 (11.70)	15.56 (85.2)	13.36 (73.0)
Female	80.31	0.93 (4.72)	1.23 (6.24)	2.80 (14.22)	2.50 (12.70)	17.50 (88.9)	15.61 (79.3)

Note : Number in parenthesis shows the percentage of each component per dried matter

Table 4. Difference of proximate components of "Suketodara" (*Theragra chalcogramma*) meat by size

Items / Size	Water content (%)	Crude fat (%)	Crude ash (%)	Total-N (%)	Pure protein-N (%)	Crude protein (%)	Pure protein (%)
L=45 *cm* W=910 *g*	81.15	0.73 (3.87)	1.21 (6.43)	2.54 (13.48)	2.36 (12.50)	15.88 (84.2)	14.75 (78.3)
L=62 *cm* W=1,850 *g*	80.71	0.84 (4.35)	1.18 (6.12)	2.65 (13.72)	2.41 (12.50)	16.56 (85.8)	15.06 (78.0)

Note : Number in parenthesis shows the percentage of each component per dried matter

As seen in Table 2, the amount of water-content in the raw sample of young fish meat was larger than in that of adult fish meat. On the contrary, all the amounts of other components in young fish meat were smaller than those of adult fish meat. Especially, it is noticeable that the amounts of fat and pure protein in adult fish meat were larger than those of young fish meat, when those amounts were compared with each other in the percentage to the dried matter of samples. From those data, it is clear that there are some differences in the amounts of chemical components according to age.

As seen in Table 3, there were differences between amounts of water-content, fat and protein. The amount of water-content in the raw female fish meat is less than that of male fish of almost the same age. The amounts of fat and protein in the female were larger than those in the male. The relations noted above were the same when comparison was made in the percentage to the dried matter of samples. These findings may be due to the accumulation of those components by female fish before the spawning.

As seen in Table 4, there are some differences of chemical components between the large sized fish (62 cm in length, 1,850 g weight) and the small sized fish (45 cm in length, 910 g weight). Both fishes were female. The amount of water-content in the raw sample of the larger fish was much smaller than that of the smaller fish. On the contrary, the amount of fat in the larger fish meat was much greater than that in the meat of the smaller fish.

Generally, the smaller fish is considered to be young, so the difference by size is also considered to be difference by age. At any rate, there are some difference between the larger fish or adult fish and the smaller or young fish.

3. Nitrogen distribution and amino acids composition of "Suketodara" meat and water-extractives

(1) Nitrogen distribution of raw meat of "Suketodara"

1) Experimental method

Fresh "Suketodara" was filleted and the raw meat removed from the skin, was homogenized; nitrogen distribution was determined by Van Slyke's method.

2) Results

The results of the determination of nitrogen distribution of the raw meat are shown in Table 5.

As seen in Table 5, about 29 % of the total nitrogen is basic amino-total nitrogen, about 53 % is mono amino-total nitrogen, and about 12 % is amide-N.

In basic nitrogen, of which about 10 % was arginine-N and lysine-N.

(2) Nitrogen distribution of water-extractives of "Suketodara" meat

1) Experimental method

Fresh "Suketodara" was filleted and the raw meat removed from the skin, was homogenized. Three hundred cc of distilled water was added to 100 g of the homogenized meat and the mixture was heated at 45° ~ 50°C with agitation for one hour. Trichloracetic acid was added to the extracted solution to precipitate protein. The precipitated protein was removed by filtration, and the separated solution was evaporated in vacuum. Thus the water-extractive was obtained. This extractive was used for determination of nitrogen distribution, after hydrolysis by 20 % HCl solution.

Table 5. Nitrogen distribution of the meat of "Suketodara" (*Theragra chalcogramma*)

Fishes / Fraction	"Suketodara" (Alaska pollack) % in total-N	"Hokke" (Atka mackerel) % in total-N	"Surume-ika" (squid) % in total-N
Total-N	100.00	100.00	100.00
20% HCl insoluble-N	1.41	1.15	—
20% HCl soluble-N	98.59	98.85	—
Amide-N	11.82	5.36	4.09
Humine-N	3.90	5.14	3.33
Basic total-N	28.71	26.13	39.70
Arginine-N	10.52	8.65	20.00
Histidine-N	6.73	8.71	8.74
Lysine-N	10.40	7.85	10.07
Cystine-N	1.06	0.91	0.92
Basic amino-N	16.34	13.83	18.90
Mono amino-acid total-N	52.56	56.56	52.80
Mono amino-acid amino-N	51.31	46.22	43.00

2) Results

Results of investigation of the nitrogen distribution are shown in Table 6.

Table 6. Nitrogen distribution of the water-extractive from "Suketodara" (*Theragra chalcogramma*) meat

Fishes / Fraction	"Suketodara" (Alaska pollack) % in total-N	"Hokke" (Atka mackerel) % in total-N	"Surume ika" (squid) % in total-N
Total-N	100.00	100.00	100.00
20% HCl insoluble-N	0.88	0.79	—
20% HCl soluble-N	99.12	99.21	—
Amide-N	8.69	0.13	2.85
Humine-N	24.90	26.21	15.71
Basic total-N	31.09	32.49	40.04
Arginine-N	12.19	24.63	—
Histidine-N	—	—	—
Lysine-N	—	—	—
Cystine-N	0.85	1.57	2.44
Basic amino-N	5.19	7.13	—
Mono amino-acid total-N	32.12	38.41	41.19
Mono amino-acid amino-N	19.08	35.12	9.44

As seen in Table 6, about 31 % of the total amount of extractive nitrogen (non-

protein-N in the extractive obtained from the fish meat by use of hot water) was basic amino-total nitrogen, and almost the same amount (about 32 %) was mono amino-total nitrogen. In basic amino-total nitrogen, arginine-N showed about 12 %, but other form of nitrogen could not be estimated, because the total amount of nitrogen in form of bases was too small.

About 25 % of the total nitrogen of the extractive was humine nitrogen.

(3) Amino acids of the "Suketodara" meat and the water-extractive

1) Experimental method

The HCl-hydrolyzates of the raw "Suketodara" meat and the water-extractive were used for two dimensional paper chromatography. The developing reagents were phenol with 10 % water, and a mixed solution of lutidine, anilin and water (65 : 7 : 28); the revealing reagent was ninhydrin-butanol solution. Revealed amino acids were identified.

2) Results

In the raw "Suketodara" meat, aspartic acid, glutamic acid, glycine, alanine, arginine, lysine, ornithine, cystine, threonine, tyrosine, leucine, histidine were identified. The kinds of amino acids identified in the water-extractive of the "Suketodara" meat were almost the same as those of the raw "Suketodara" meat.

Consequently, the nitrogen distribution and the amino acids composition of the "Suketodara" meat can be stated to be almost the same as those of other fish meats[1].

II. DECOMPOSITION OF THE "SUKETODARA" MEAT

It has been known that when the freshness of "Suketodara" meat falls, the elasticity of the fish paste products ("Ashi" of "Kamaboko") decreases[2].

The reason for this phenomenon begins recently to understand from the increased knowledge in the field of protein chemistry. But, here, it is important to know the velocity of decomposition of the "Suketodara" meat for solving the problems of employment of this fish for various products.

The authors have tried to calculate the decomposition velocity constant, temperature coefficient and temperature constant from the data obtained when the "Suketodara" meat was left and allowed to decompose.

1. Changes of the amounts of volatile basic nitrogen when the "Suketodara" meat is left

(1) Experimental method

From fresh "Suketodara" body, head and viscera were removed. Each two carcasses were left as they are at 35° ± 1°C, 25° ± 1°C and 10° ± 1°C, respectively. And the fish meat was allowed to decompose aerobically. At certain definite intervals of leaving time, a part of each fish body was taken off; on such portions the amount of

volatile basic nitrogen (V.B.-N) was estimated by Weber and Wilson's method. From the amounts of V.B.-N estimated, the relations among the amount of V.B.-N produced, leaving time and leaving temperature were determined.

(2) **Results**

At the respective leaving temperatures, the variations in amount of V.B.-N in the fish meat were as shown in Fig. 2.

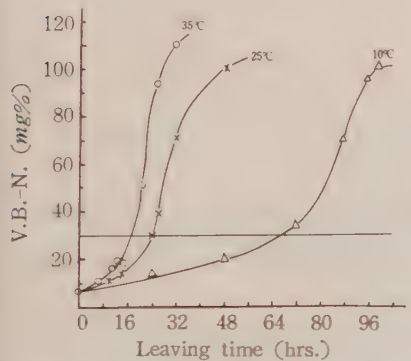


Fig. 2. Changes in the amount of volatile basic nitrogen during the leaving of carcasses of the "Suketodara" (*Theragra chalcogramma*) at various temperatures

When carcasses were left at higher temperature, the amount of V.B.-N increased rapidly. On the contrary, at comparatively lower temperature, the amount of V.B.-N increased slowly.

2. Calculations of decomposition velocity coefficient (K), temperature coefficient (Q_{10}) and temperature constant (B)

From data obtained as shown in Fig. 2, the relation between "y" and "$\log \frac{y}{A-y}$ (here, "y" is the producing amount of V.B.-N at each time "t" of the estimation, "A" is the maximum amount of V.B.-N produced) was calculated and graphed in Fig. 3.

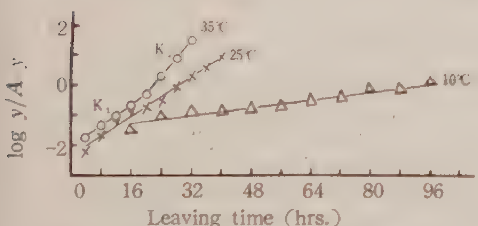


Fig. 3. Relation between the value of $\log \frac{y}{A-y}$ and time "t"

As seen in Fig. 3, the relation between "$\log \frac{y}{A-y}$" and "t" is linear.

Therefore, the following equation is obtained after Kimata[3].

$$\log \frac{y}{A-y} = Kt + C \quad \cdots\cdots\cdots\cdots\cdots(1)$$

Here, C is a constant.

In this equation of the monomolecular autocatalytic reaction, the value of "K" is decomposition velocity coefficient (V.B.-N producing velocity coefficient).

From equation (1), the values of "K" at various leaving temperatures were calculated as shown in Table 7.

Table 7. Value of "K" of "Suketodara" (*Theragra chalcogramma*) meat at various leaving temperatures

Temp. (°C)	$K \times 10^3$
35°	$K_1 = 89$, $K_2 = 101$
25°	79
10°	28

As seen in Table 7, the higher the leaving temperature, the larger the value of "K". At 35°C leaving temperature, that the value of K_1 is less than K_2, may be due to the fact that the decomposition of the meat is slow at initial stage, but becomes rapid

from the middle stage.

After Ōya[4], from equation (2) of Arrhenius' fundamental equation, temperature constant (B) and temperature coefficient (Q_{10}) were derived as follows:

$$\log K + \frac{B}{R} \cdot \log e \frac{1}{T} = \text{const.} \quad \cdots\cdots (2)$$

$$B = \frac{a}{\log e} \cdot R = \frac{a}{0.4343} \cdot 1.985 \quad \cdots\cdots (3)$$

$$\text{here, } a = \frac{B}{R} \cdot \log e$$

$$\log Q_{10} = \frac{B}{R} \cdot \log e \left(\frac{1}{T} - \frac{1}{T+10} \right) = a \cdot \frac{10}{T(T+10)} \quad \cdots\cdots (4)$$

Here, "K" is the decomposition velocity coefficient at T°K; R is gas constant and it is 1.985 cal.

Here, the values of $\log K \times 10^3$ at respective temperatures were plotted on ordinate and the reciprocal values of the absolute temperature of the estimation, $\frac{1}{T}$, were plotted on abscissa; Fig. 4 was obtaind.

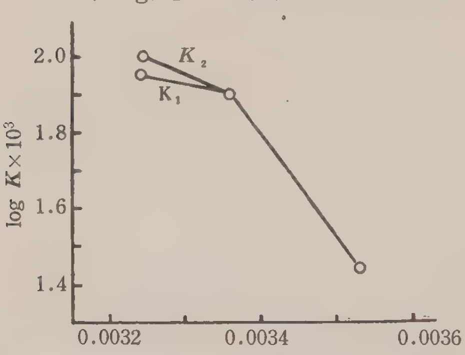


Fig. 4. Relation between the value of "log K" and "$\frac{1}{T}$"

As seen in Fig. 4, the relation is linear in the range between two different temperatures. The degree of the inclination of the straight lines is shown as "a" value in equations (3) and (4). Here the values of "B" and "Q_{10}" are obtained at each range of temperatures as shown in Table 8.

Table 8. Values of temperature coefficient, "Q_{10}", and temperature constant, "B", of "Suketodara" (*Theragra chalcogramma*) meat

Temp. range		Q_{10}	B
10° ~ 25° C		2.2	11,900
25° ~ 35° C	K_1	1.3	4,100
	K_2	1.0	1,800

The value of "K" of "Suketodara" meat at various temperatures is compared with those of other marine creatures obtained by Tanikawa[5] as shown in Table 9.

As seen in Table 9, the "Suketodara" meat is easily decomposable even at low temperature.

The values of "Q_{10}" and "B" of "Suketodara" meat at 25°C in comparison with those of other marine creatures are shown in Table 10.

As seen in Table 10, at comparatively higher storing temperature, "Suketodara" meat is more easily decomposable than other fish meat.

Thus the "Suketodara" meat is decomposable, so care should be taken in the handling

Table 9. Values of "*K*" in "Suketodara" (*Theragra chalcogramma*) meat compared with those of other marine creatures ($K \times 10^3$)

Fishes \ Temp. range	10° C	14° ~ 16° C	24° ~ 28° C	33° ~ 37° C
Sea cucumber[5a)]	—	17	28	29
crab[5b)]	—	—	74	91
Atka mackerel[5c)]	—	22	36	60
mackerel[5d)]	—	30	99	114
saury[5e)]	—	27	30	48
squid[5f)]	—	—	103	151
"Suketodara"	28	—	79	{89, 101}

Table 10. Vaules of "Q_{10}" and "*B*" of "Suketodara" (*Theragra chalcogramma*) meat and those of other fish meat

Fishes	Temp. range	Q_{10}	*B*
Atka mackerel[5c)]	above 25°C	1.5	8×10^3
	below 25°C	3.5	21×10^3
mackerel[5d)]	above 25°C	1.2	3×10^3
	below 25°C	3.6	22×10^3
"Suketodara"	above 25°C	1.3	4×10^3
	below 25°C	2.2	12×10^3

of this fish. Before the processing, it must be stored at low temperature.

3. Calculation of initial decomposition velocity coefficient

It is rather important to know the initial decomposing value of "*K*".

Next, the authors have determined the relation between the storage time and amount of V.B.-N produced until that amount reaches to 30 mg% (incipient putrefaction). (Ref. Fig. 2).

Up to the value of 30 mg%, the curve showing the relation between the storing time and amount of V.B.-N produced is almost hyperbolic. For example, it requires 17 hours at 35°C, 25 hours at 25°C and 68 hours at 10°C for the amout of V.B.-N to reach 30 mg%. But when the amount of V.B.-N in "Suketodara" meat reaches 30 mg%, the meat smells putrefactive, so the limit of the freshness should be set at a rather less amount of the V.B.-N (the authors consider it is 20 mg%).

As follows, the authors have devised an equation (5) applicable for estimating the beginning of incipient putrefaction.

$$V = pt^2 + b \quad \cdots\cdots (5)$$

Here, "*V*" is the amount of V.B.-N in the fish meat after "*t*" hours, "*b*" is a constant (in this case it is the amount of V.B.-N, when the storing time is 0); "*p*" is the coefficient of the velocity of decomposition in the initial stage.

The values of "*p*" obtained from equation (5) are compared with those from other fish meat as shown in Table 11.

Table 11. Values of "p" of "Suketodara" (*Theragra chalcogramma*) meat compared with corresponding values of other fish meat

Temp. \ Fishes	salmon	squid	"Suketodara"
35°C	0.367	0.082	0.075
25°C	0.023	0.031	0.034
10°C	0.013 (15°C)	0.003	0.006

As seen in Table 11, as the value of "p" is larger at 25° or 10°C than that of other fish meat, the "Suketodara" meat is seen to be easily decomposable in the initial stage.

4. Change of histological aspects during the falling of freshness of the "Suketodara" meat

When the fresheness of the "Suketodara" meat falls, the chemical properties of the meat change. This is accompanied by histological change. Here the authors have attempted to observe the histological aspect during the falling of the freshness of the "Suketodara" meat.

(1) Experimental method

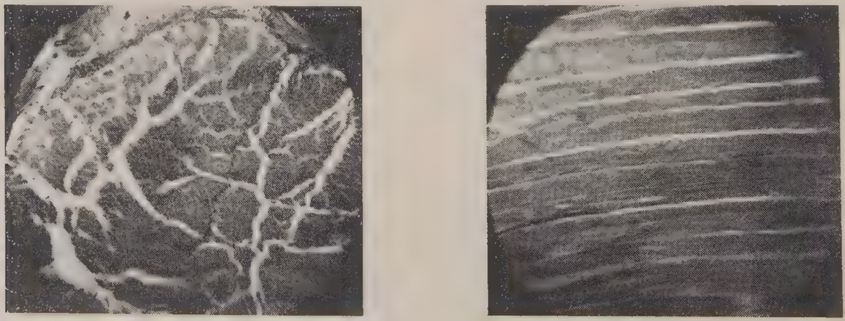
i) For the making of histological preparates as samples, fresh "Suketodara" meat and unfresh fish meat, of which V.B.-N value was 50 mg% were used. From both sorts of fish bodies, a block of a part of back muscle meat ($0.5\times0.5\times1.0cm^3$) was taken out. Those blocks were fixed with Bouin's solution. Then they were dehydrated with alcohol as usual, and finally imbedded in paraffin. Thus treated meat was sliced in 10μ (or 15μ) thicknesses by a microtome. Each slice was dyed by Delafields' haematoxyline staining method and imbedded in balsam to make a parmanent preparate.

ii) Microscopic observation

Microscopic observations were photographed as shown in Fig. 5-1, Fig. 5-2 and Fig. 5-3.

Fig. 5-1 and Fig. 5-2 show photographs of fresh "Suketodara" meat; Fig 5-3 shows the model chart of the same fresh meat.

Figs. 6-1, 6-2 and 6-3 show the results on unfresh meat.



Figs. 5-1,2. Section of fresh "Suketodara" (*Theragra chalcogramma*) meat muscle

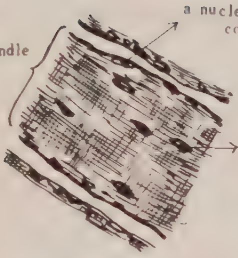
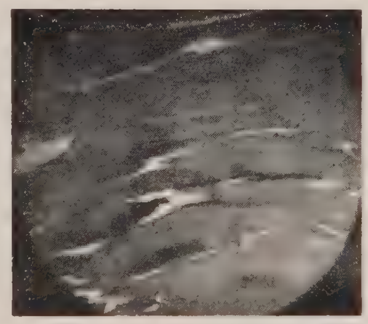


Fig. 5-3. Model chart of fresh "Suketodara" (*Theragra chalcogramma*) meat muscle

As to fresh "Suketodara" meat, observations were made from two directions parallel or perpendicular to muscle fiber bundles. A muscle fiber bundle consists of several hundred muscle fibers which were bound twisting. This muscle fiber is one unit of the cell, and is composed of myofibril. A muscle fiber is surrounded by connective tissue having





Figs. 6-1,2. Section of unfresh "Suketodara" (*Theragra chalcogramma*) meat muscle

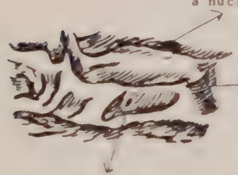


Fig. 6-3. Model chart of unfresh "Suketodara" (*Theragra chalcogramma*) meat muscle

nuclei. Many muscle fibers are enveloped by a muscle sheath (sarcolemma) making a muscle fiber bundle. Meat muscle is composed of transversely striated muscle, so nuclei are seen in meat muscle tissues. There are spaces between the muscle fiber bundles which are filled with water, body fluid, blood vessels and lymphatic vessels.

As to unfresh "Suketodara" meat, different histological observations from those of fresh "Suketodara" meat were obtained. The meat became opaque. Striae became not clear. Cell nuclei in the connective tissues were remarkably deformed. This is considered due to the phenomena caused by karyorrhexis, karyolysis and pycnosis. At every part the muscle sheaths dissolved, and they are put one upon another. This may be due to the deformation of connective tissues which are binding muscle fibers. For these phenomena Kawabata[6] has given the name "waxy degeneration" of muscle fiber. The degrees of the deformation of tissues differs with the leaving time and temperature. Concomitant with the falling of the freshness of the meat and the deforming of the muscle bundles, fluid matter escapes from the inter-spaces, and the proteins of the meat may denaturalize.

5. Change in the amount of bound water during the falling of freshness of "Suketodara" meat

The degree of the denaturation of the meat protein may be judged by estimating the amount of bound water in the meat.

The authors have estimated the amount of bound water by the vapour tension method.

(1) Experimental method

Fresh "Suketodara" bodies were left in ice box (10° ± 2°C) and the samples of meat having various degrees of freshness were obtained as shown in Table 12.

Table 12. The samples used in the experiment of hydration of decomposed "Suketodara" (*Theragra chalcogramma*) meat

Items \ Leaving times	0 day	3 days	6 days
Water content (%)	81.18	81.80	81.52
pH	6.2	6.6	6.8
Volatil basic-N (mg%)	5.34	50.06	83.34
Total-N (%)	2.35	2.51	2.68
Pure protein-N (%)	2.26	2.25	1.72
Water soluble-N (% in total-N)	37.2	34.2	27.9
0.5 M NaCl-soluble-N (% in total-N)	46.0	36.4	34.4

The samples above described were employed for the estimation of the amount of bound water by means of the vapour tension method[7]; the water-content "g" —relative vapour pressure ($p/p_\circ$) curve was obtained.

(2) Results

The experimental results are shown in Fig. 7.

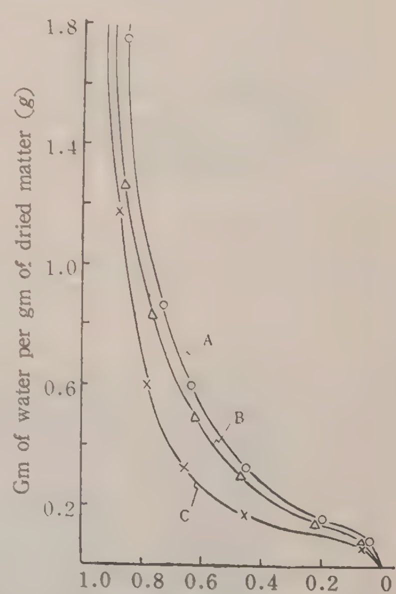


Fig. 7. Water-content (g) — relative vapour pressure ($p/p_\circ$) curves of fresh and unfresh "Suketodara" (*Theragra chalcogramma*) meat

Here, "g" is grams of water per gram of the dried matter, and "p" is the vapour pressure of water containd in sample at the estimating temperature "t°C", and "$p_\circ$" is the same of pure water at the same temperature. As seen in Fig. 7, with the falling of freshness the curve moved to the left. This shows the increase of the relative vapour pressure ($p/p_\circ$) in the entire range of water-content "g". That is to say, the curve shows the increase of water-activity "a" of the water contained in the sample. The amount of bound water is different more or less according to the properties of the kinds of proteins, the presence of salts, estimating temperatures, etc. But it is generally considered that the water of which the water-activity "a" is 0.6 ~ 0.8, is a colloid-chemical bound water[7]. In Fig. 7, the differences between curves (A) and (B), or (B) and (C) show the decrease in the amount of bound water with the falling of the

freshness of the "Suketodara" meat. Here the value of the difference, $\Delta\bar{F}$, between the differential molal free energy of pure water and that of water in the sample at the same temperature is calculated by the following equation, according to Lewis and Randall[7].

$$\Delta\bar{F} = F^{\circ} - \bar{F} = -RT\ln p/p_0 \quad \cdots\cdots\cdots\cdots\cdots\cdots (6)$$

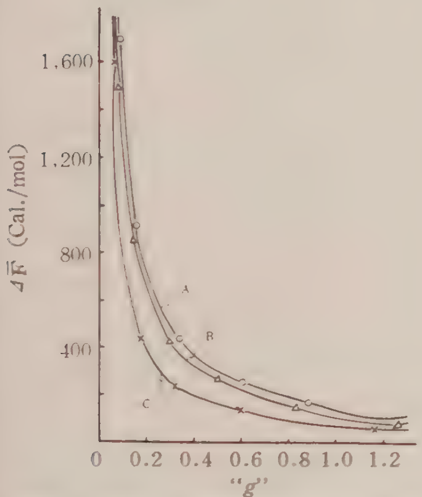


Fig. 8. Relation between the values of "*g*" and $\Delta\bar{F}$ of fresh and unfresh "Suketodara" (*Theragra chalcogramma*) meat

Here, F° is the differential molal free energy of pure water at T°K, $\bar{F}$ is that of water in the sample at the same temperature and R is gas constant.

Fig. 8 shows the relation between the values of "*g*" and $\Delta\bar{F}$.

As seen in Fig. 8, the value of $\Delta\bar{F}$ at a definite amount of water-content increased with the falling of the freshness of sample meat used. This shows the decrease of affinity of water to the meat protein with falling of the freshness, in which case the denaturation of the meat protein occurs.

III. CHEMICAL PROPERTIES OF "SUKETODARA" MEAT PROTEIN

1. Dissolution of "Suketodara" meat

It is important to know the solubility of the "Suketodara" meat by water, acid or alkaline solutions, because when this fish meat is used as the raw material of various products, especially for fish paste ("Kamaboko" or "Chikuwa"), the solubility influences the quality of the fish paste.

Next, the authors undertook to determine the solubility by various solutions.

(1) Experimental method

After fresh "Suketodara" had been filleted, the meat was removed from the skin and homogenized.

To 10 g of the homogenized meat, 200 cc of dist. water was added and the solution was stirred for 30 minutes, then centrifuged (3,000 r.p.m. for 30 minutes). An upper transparent liquid was obtaind. Five cc of the liquid was used for the estimation of the amount of dissolved nitrogen by Kjeldahl's method.

The amount of nitrogen dissolved out by water was 30.6 % of the total amount of nitrogen in sample meat.

Next, similarly, the homogenized "Suketodara" meat was used for the estimation of

the solubilities of the meat by various salt, alkaline or acid solutions.

With each 10 g samples of the homogenized meat, 50 cc each of NaCl, KCl, NaOH, H_2SO_4, HCl and CH_3COOH solutions having various concentration was put into tall glass bottles, respectively. The bottles were shaken frequently and were left for 24 hours.

After the leaving, the upper transparent liquid was obtained by centrifugation. Five *cc* of the liquid was used for the estimation of amount of dissolved nitrogen.

The solubities (*S*) were calculated by the following equation (7)

$$S = \frac{a \times 100}{\text{total amount of nitrogen in 1 g of the sample}} \ (\%) \quad \cdots\cdots\cdots\cdots\cdots (7)$$

Here, "*a*" is the amount of nitrogen in 5 cc of the upper transparent liquid. The amount of nitrogen in 1 g of the sample was 24.45 mg.

(2) **Results**

Results obtained are shown in Fig. 9.

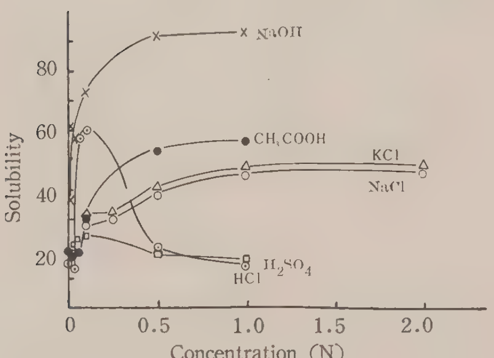


Fig. 9. Solubility of "Suketodara" (*Theragra chalcogramma*) meat by various salt, alkaline or acid solutions

The amount of nitrogen in the "Suketodara" dissolved by water was 30.6 % as described above.

As seen in Fig. 9, the solubility of the meat by NaCl solution differs according to the concentration, but it ranged 30 ~ 45 %. The solubility by KCl solution has almost the same tendency with NaCl, but somewhat larger.

On the other hand, the solubility by NaOH solution is large, 90 %. Differently, the solubilities by acids vary with the kind. For example, there is remarkable difference between hydrochloric acid and sulfuric acid. Okada *et al*.[9] or Fujii[1] have explained this difference as follows: hydrochloric acid acts on proteins as a mono-basic acid, while sulfuric acid acts as a di-basic acid. The difference in the case of the "Suketodara" meat also can be explained in the same manner.

The solubility of the "Suketodara" meat in acetic acid increased in accordance with the increase of the concentration.

In general, the solubility by alkaline solution did not decrease with the increase of the concentration below 1 N, differing from acid solution. The reason why such a difference exists may be explaind as follows: in acid solution in high concentration the protein of the fish meat coagulates, contrarily in alkaline solution the meat protein is hydrolyzed. From the results obtained, the "Suketodara" meat can be said to be dissolved by water or NaCl solution, when the meat is soaking in water or NaCl solution during the processing of the fish paste ("Kamaboko" or "Chikuwa").

2. Isoelectric points of protein of the "Suketodara" meat

The "Suketodara" meat consists of a few kinds of protein, therefore the solubility of the meat by various solvents differs.

Next, the authors have tried to determine the isoelectric points of proteins which are prepared from extracting meat by means of water or NaCl solution.

(1) Experimental method

Fresh "Suketodara" was filleted and the meat was removed from the skin, and then homogenized. The homogenized meat was extracted by water or 0.6M NaCl solution. Thus water soluble protein or NaCl soluble protein solutions were obtained. Each 10 cc of those water soluble or NaCl soluble protein solutions was added to each 40 cc of the buffer solutions having various pH values which were prepared from N/25 NaOH and N/25 HCl solutions. After the solutions had been left for 24 hours, the resultant precipitate was filtered. The pH value and viscosity of the filtrate were determined. The relative viscosity (η/η_0) was measured by Ostwald's viscosimeter. The pH value of the solution which indicates the minimum relative viscosity is regarded as the isoelectric point of the protein. On the other hand, the total amount of nitrogen in the precipitated substance noted above was estimated, and the pH value of which the maximum amount of nitrogen, was determined and regarded as the isoelectric point of the protein. Comparing the isoelectric points which were determined by the viscosity method or by the method of the estimation of the total amount of nitrogen in the precipitated substance, the two isoelectric points were found to be in agreement as shown in Figs. 10 and 11.

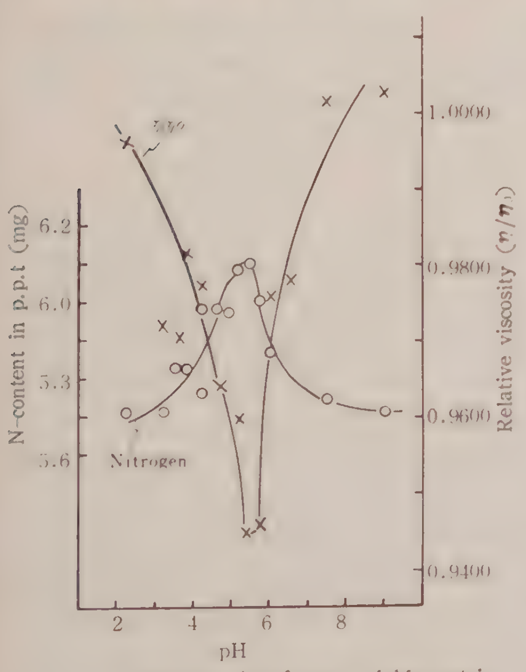


Fig. 10. Isoelectric point of water soluble protein of "Suketodara" (*Theragra chalcogramma*) meat

(2) Results

As seen in Figs. 10 and 11, the isoelectric point of water soluble protein of the "Suketodara" meat was pH 5.2 ~ 5.4, and that of NaCl solution soluble protein was pH 5.0 ~ 5.2. The reason why the isoelectric point has some range is due to the fact that the protein is not a single component, but consists of several components.

The isoelectric point is different according to the kinds of fish or constituent proteins. Tadokoro[1] has examined cod, sardine or flat fish meats, and

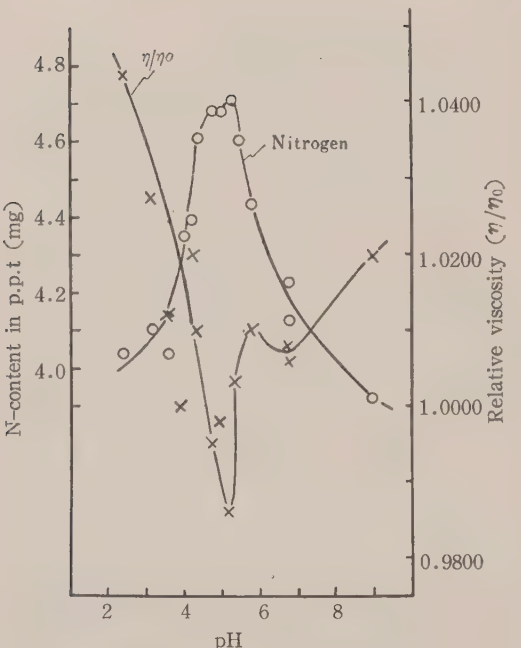


Fig. 11. Isoelectric point of 0.6M NaCl solution-soluble protein of "Suketodara" (*Theragra chalcogramma*) meat

Takagi[11] has also examined "Nibe" (*Nibea mitsukurii*) meat; they made it clear that the isoelectric point of fish meat protein is different by the kinds of fish constituent protein and method of preparation of the protein.

The "Suketodara" meat is also constituted of several proteins.

3. The phenomenon of flow birefringence of the extractive of the "Suketodara" meat

Among several kinds of proteins in the "Suketodara" meat, "myosins" (including actomyosin, L-myosin and actin) are considered to have an intimate relation with the quality of the fish paste ("Kamaboko") product.

The authors undertook to observed the flow birefringence in the solutions of water soluble protein, Weber's solution-soluble and NaCl solution-soluble proteins by the use of the same apparatus employed in studying the extracts of *Stichopus japonicus*[12].

(1) Experimental method

Fresh "Suketodara" was filleted, the meat was removed from the skin and homogenized. The homogenized meat was used for the sample. To 20 g of the sample meat, 200 cc of distilled water was added. After 30 minutes' stirring of the mixture, it was centrifuged. The flow birefringence of the upper clear liquor thus obtained was observed. The total amount of nitrogen in the same upper clear liquor was estimated, and the dissolved ratio (%) of the nitrogen to the total nitrogen in the raw meat was presented as the solubility. To the residue of the 1st extraction, 200 cc of distilled water was added and the 2nd extraction was carried out. Intensity of birefringence and the total amount of nitrogen were estimated for the 2nd extract. The extractions were continued until the 5th extraction. Extraction was also done by the use of Weber's solution (which consists of KCl, $NaHCO_3$ and Na_2CO_3 in the proportion of 0.6, 0.04 and 0.01 Mol respectively). Thus the dissolution of myosins was detected.

(2) Results

Results obtained are shown in Tables 13 and 14.

Table 13. Flow birefringence and amount of nitrogen dissolved in water extracted solution of "Suketodara" (*Theragra chalcogramma*) meat and appearance of the sediment

No. of extraction	Soluble-N (% in total-N)	S. B.	Appearance of sediment
S_1	26.45	—	
S_2	9.35	—	Did not effuse by decantation
S_3	7.42	—	Softening of meat, upper layer of sediment flowed out by decantation
S_4	4.92	—	Further softening, ditto
S_5	Trace	—	Ditto
Residue	46.90		

Table 14. Flow birefringence and amount of nitrogen dissolved in the extract of "Suketodara" (*Theragra chalcogramma*) meat obtained by use of Weber's solution and appearance of the sediment

No. of extraction	Soluble-N (% in total-N)	S. B.	Appearance of sediment
S_1	37.74	—	
S_2	12.76	—	Did not effuse by decantation
S_3	5.83	—	Softening of meat, did not effuse
S_4	Trace	—	Slight coagulation of meat
S_5	Trace	—	Ditto, coagulation of meat
Residue	40.60		

In the case of the 1st extraction of the "Suketodara" meat with water, no pattern of flow birefringence appeared. After the 5th extraction, the residue was extracted with Weber's solution. But this extract also did not show flow birefringence. On the other hand, in the extraction of the meat with Weber's solution, even in the case of the 1st extraction flow birefringence was not seen. After the 5th extraction, the residue was extracted with the same solution. But this extract also did not show flow birefringence. In the case of the extraction with 0.6 Mol NaCl solution, every extraction also showed no pattern of flow birefringence.

That is to say, the extractions of "Suketodara" meat with water, 0.6 M NaCl solution or Weber's solution all showed no pattern of flow birefringence. But the amount of nitrogen in the 1st extraction with Weber's solution was larger than that in the 1st extraction with water. After the 2nd extraction with Weber's solution or water, the amount of nitrogen dissolved out became small. In the extraction of the meat with Weber's solution, the amount of nitrogen dissolved out showed only trace after the 4th extraction.

According to Okada *et al* [illegible], the extracted solution of fish meat muscle with 0.6 Mol KCl solution shows flow birefringence (S.B. +).

This is due to the reason that myosins (including actomyosin, L myosin and actin)

dissolve easily into salt solution, and the fact that those filamentous molecule coordinate in one definite direction by flowing. On the contrary, the extracted solution of fish meat muscle with water does not show flow birefringence. (However, there are some exceptions. For example, the water-extraction of squid or sea cucumber meat shows flow birefringence because of their characteristics of histological properties or proteins[12)13)]).

In the present experiments, considering that the amount of nitrogen dissolved with 0.6 Mol NaCl solution or Weber's solution was larger than that with water, it is suggested that myosins which are soluble by salts solutions shall be dissolved out. Therefore, the fact that 0.6 Mol NaCl or Weber's extracted solution did not show flow birefringence is considered to be peculiar. This is considered explicable as follows: the amount of the dissolved nitrogen from the "Suketodara" meat with salts solutions might be too small to show flow birefringence by use of the apparatus employed of which the accuracy was limited. Furthermore, peculiarities of myosins in the "Suketodara" meat, for example the quantitative ratio of actomyosin, L-myosin and actin, are also considered to be involved in the explanation. According to Okada *et al.*[13)], when the 0.6 M KCl extracted solution of fishes meat, or fishes' meat themselves are frozen, the showing of flow birefringence disappears in a comparatively short time. This may be explained by the denaturation of the protein by freezing.

As examined in the previous experiment, Article II above, the freshness of the "Suketodara" meat falls easily, therefore the denaturation of meat protein of the "Suketodara" also occurs easily. So the elasticity of the fish paste products becomes small. Considering together those facts with the peculiarities of the "Suketodara" meat which are observed in the phenomenon of flow birefringence, it is especially important to examine those peculiarities of this fish meat in relation to the properties of myosins in the meat.

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TWO SPONGES OBTAINED BY THE TRAINING SHIP "OSHORO MARU" IN THE EASTERN BERING SEA

By

Senji TANITA

(Tohoku Regional Fisheries Research Laboratory, Shiogama, Miyagi Pref.)

The sponges upon which this report is based were collected by the Training Ship "Oshoro Maru" of the Faculty of Fisheries, Hokkaido University during the course of the North Pacific Oceanographic Expeditions in 1955 and 1956 in the Northern Seas. This collection contains only two species, but affords an interesting addition to knowledge of the northern deep sea sponge-fauna. By the kindness of Mr. K. Kobayashi of Hokkaido University, the writer had the opportunity to examine and describe them. Here he wishes to express his hearty thanks to the "Oshoro Maru" extention committee, especially to Mr. Kobayashi for his kindness.

Class Demospongiae
 Order Halichondrina
  Family Axinellidae
   1) *Phakellia ventilabrum* (JOHNSTON)
  Family Halichondriidae
   2) *Halichondria oshoro* n. sp.

Description of Species

1) *Phakellia ventilabrum* (JOHNSTON)

(Figs. 1 and 2)

Halichondria ventilabrum, JOHNSTON (1842) p. 107, Pl. 7.

Phakellia ventilabrum, BOWERBANK (1864) Vol. 1, p. 186; Vol. 2, p. 122; Vol. 3, p. 57, Pl. 22, figs. 1—7: SCHMIDT (1870) p. 61: RIDLEY and DENDY (1887) p. 170, Pl. 35, fig. 3: VERRILL (1873) Vol. 1, p. 440; Vol. 2, p. 413: WHITEAVES (1874) p. 9: LAMBE (1894) p. 124; (1896) p. 192, Pl. 2, fig. 3; (1900) p. 24; p. 161; p. 277: TOPSENT (1928) p. 174: HENTSCHEL (1929) p. 897; p. 974: ARNDT (1935) p. 90, fig. 191.

Phakellia robusta, BOWERBANK (1866) p. 120; p. 54, Pl. 21, figs. 13—17.

The sponge obtained by the trawl of the "Oshoro Maru" in the Bering Sea at 57° 32′ N., 171° 41′ W. is assigned to this species.

The specimen is cup-shaped with a short, rather slender stalk. It is composed of two individuals in contact side by side and attached on a carapace of a maiidaean crab, *Acanthophrys sp.*, with stalks. The larger individual is 85 mm high, 60 mm across while the smaller is 65 mm in height and 35 mm in diameter of the cup. The latter is strongly compressed laterally.

The wall of the larger sponge is about 10 mm in thickness; the upper margin of the body is strongly undulated and finely plicated. The surfaces of the outer and inner portions of the cup are nearly even, but not smooth to the touch because of the projections



Fig. 1. *Phakellia ventilabrum* (JOHNSTON) × 2/3

of the terminations of the primary fibres of the skeleton. This hispidation is visible only when examined microscopically. It is produced by the extension of the primary spiculo-fibres of the skeleton, and partly by the projection of single spicules through the dermal membrane.

About ten oscula with diameters of 2~3 mm are arranged in a circle at the center of the inner portion of the cup. The inside of the cup is seen as an irregular reticulation resultant from the presence of numerous pores with diameters of less than 1 mm.

The texture of the sponge is rigid in the stalk portion and slightly elastic in the upper portion. Colour in spirit (after formalin) is pale grey, with external contamination with mud.

The skeleton is composed of spiculo-fibres; of them the primary lines are multispiculous, strongly developed, rather irregular and the secondary lines very irregular, mostly multispiculous, and very numerous.

Spicules (Fig. 2):— Smooth styles, of three kinds but not by any means sharply distinguished from one another. Large, stout styles (Fig. 2 a) are slightly curved, broadly and evenly rounded off at the base and more or less gradually and sharply pointed at the apex, 500—900μ in length and 18—20μ in thickness. Slender, long styles (Fig. 2 b) nearly straight or slightly curved, 200—800μ in length and average 5 μ in thickness.

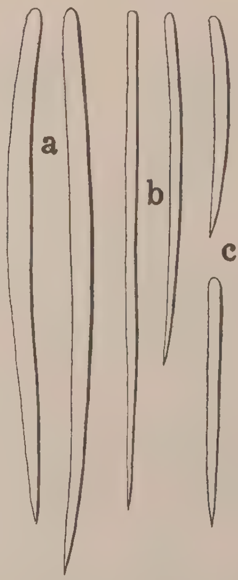


Fig. 2. Spicules of *Phakellia ventilabrum* (JOHNSTON) a: large stout styles, b: slender long styles, c: short stout styles. All × 100

Short, stout styles (Fig. 2 c) are the same in shape as the large stout ones but in size, measure 250～400×10～12μ.

Previously known Distributions: British Sea (JOHNSTON, BOWERBANK); Iceland (JOHNSTON); Shetlands (BOWERBANK); between Scotland and Faröe Islands (CARTER); south-west coast of Norway (SCHMIDT); Arctic Ocean, off Norway (VOSMAER); Baltic Sea (FRISTEDT); Gulf of St. Lawrence (WHITEAVES, LAMBE); Florida, Gulf of Mexico and Barbados (SCHMIDT); Brazil and north-east of Falkland Islands (RIDLEY and DENDY); north-east coast of U. S. A. (VERRILL); Murray Bay, south-east of Bonaventure Island (LAMBE); Hudson Bay (LAMBE); Davis Strait (LAMBE); Parages de Belle Ile (TOPSENT).

Locality :— Bering Sea, 57° 32′ N., 171° 41′ W. Depth 105 m.

Remarks: This remarkable species was originally described and figured by JOHNSTON in 1842 based on a specimen obtained from the British Seas under the name of *Halichondria ventilabrum*. Since that time it has hitherto been recorded by BOWERBANK, SCHMIDT, and other several investigators from various localities of the world. Judging from the distribution above mentioned, this species may be one of the cosmopolitan sponges. The species is an inhabitant of the deep sea as RIDLEY and DENDY (1887) have already pointed out, being common in depths over 100 fathoms.

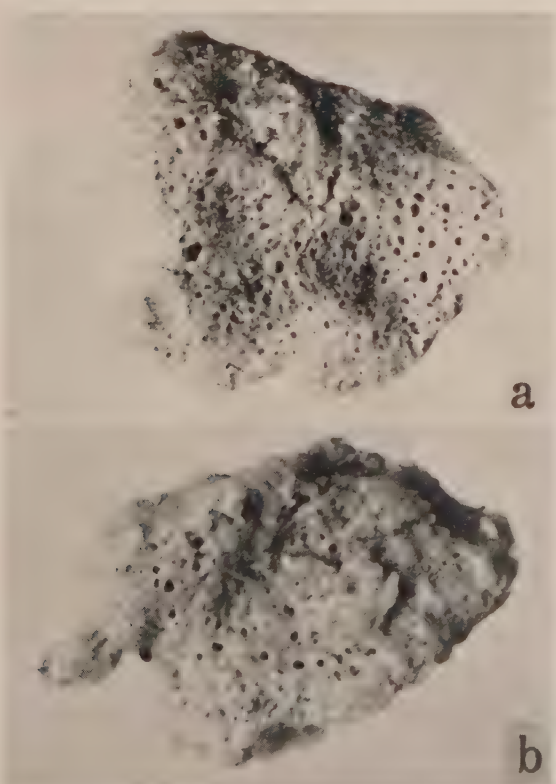


Fig. 3. *Halichondria oshoro* n. sp. × 2/3

2) *Halichondria oshoro* n. sp. (Figs. 3 and 4)

This new species is represented by eighteen large and small fragments, all which probably belong to two or three specimens. They differ considerably from one another in external appearance; some of them are massive, some cylindrical and some amorphous but never encrusting in shape. All of them were obtained by the trawl from Bristol Bay in 1955.

The largest specimen (Fig. 3 a) which the writer selected as the type of the species has a slightly compressed, slightly concave upper surface, irregular massive body, and

measures 70 mm long, 55 mm broad by 15 mm thick. The surface of the sponge is uneven, very minutely hispid, with more or less circular oscula scattered irregularly over the surface. The oscula vary in diameter from 2 mm to less than 1 mm and are very abundant. The endosome of the sponge is typical of the genus, being somewhat like bread crumbs; small caverns or chambers are surrounded by spicules in confusion. The colour is yellowish green in formalin but changes to pale grey after being preserved in alcohol, the texture is soft and fragile.

The dermal skeleton is an irregular reticulation of tangentially disposed slender oxeas, associated with the outer ends of endosomal spicules whose meshes are abundantly filled with slender oxeas.

The main skeleton halichondroid, with loose, ill-defined fibre running more or less vertically to the surface, with a tendency to branch and anastomose. Between the bundles numerous loose spicules are scattered lying without order.

Spicules (Fig. 4):— Oxea of two distinct categories; large, stout and small, slender ones. Oxeas of endosome (Fig. 4 a) very slightly curved, sharply pointed at both ends, measuring 450～580×10～17μ. Oxeas of dermal portion (Fig. 4 b) slender, slightly curved, tapering to sharp ends, measuring 250～400×4～5μ.

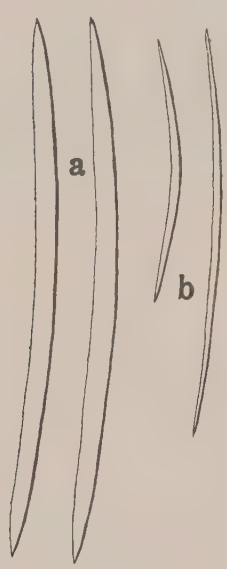


Fig.4. Spicules of *Halichondria oshoro* n. sp. a: endosomal oxeas, b: dermal oxeas. All × 140

Locality:—Bristol Bay.

Remarks:— This species has two kinds of oxeas and thus appears to be more closely allied to ***Halichondria fibrosa*** (FRISTEDT) or ***H. genitrix*** (SCHMIDT), than to any other members of the same genus. It differs from the allied forms, however, not only in colour, texture and external form but also in dimensions of the spicules.

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STUDIES ON THE MELOBESIOIDEAE OF JAPAN. IV

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Faculty of Fisheries, Hokkaido University

7. **Polyporolithon reclinatum** (Foslie) L. R. Mason

Pls. I—IV

Mason, 1935, p. 319, pl. 30; Tokida & Masaki, 1960, p. 497.

Syn. *Lithothamnium conchatum* Setchell et Foslie f. *reclinatum* Foslie, 1906, p. 6. *Lithothamnium reclinatum* (Foslie) Foslie, 1907, p. 14: 1929, p. 45, pl. 10, figs. 14–17; De Toni, 1924, p. 618.

Japanese name. Kasa-kinoko-ishimo (n. n.).

Habit and distribution, in literature. Hemiparasitic on *Cheilosporum frondescens, Bossiella, and Corallina.* From Vancouver Island, B. C., to California along the coast of North America.

Specimens collected. On *Pachyarthron cretaceum.* Muroran, 20 March 1960, Tokida and Masaki; 1 June 1960, Yamamoto. Shirikishinai, near Hakodate, 23 July 1960, Niihama.

The following description is based on the specimens from Muroran.

Thallus irregularly circular or elliptical small disk, sessile, often recurved and encircling host partly or completely, up to 1.8 cm long by 0.7 cm broad, 0.3–1 mm thick, attached to host by sub-penetrating base near the center of lower surface, deep reddish purple in color, polystromatic to margin; epithallium 1–3 layers of cells thick except at the very edge of the thallus where it is lacking, cells in section rectangular and 7 μ in diam. or subquadrate and 5–9 μ long by 4–7 μ broad; perithallium 0.15–1.3 mm μ thick, cells subquadrate, 10–17 μ diam., or elongated and 15–26 μ long by 8–12 μ broad, uppermost cells deeply stained with haematoxylin and anilin blue, vertically elongated and 12–30 μ long by 5–9 μ broad; hypothallium 50–230 μ thick, composed of branched cell-rows curved upward and downward, cells rectangular, 13–50 μ long by 8–13 μ broad; coalescence of cells between adjacent cell-rows in perithallium and hypothallium present; sporangial conceptacles 147–273 μ high, 290–435 μ diam., deeply embedded, roof flat or slightly convex, perforated by 25–30 pores, sporangia tetrasporic, 109–197 μ long, 45–87 μ diam., 7–10 sporangia standing on floor of each sporangium; sexual plant normally dioecious but sometimes monoecious; procarpic conceptacles slightly convex, 126–252 μ high, 147–190 μ diam., auxiliary cells carrying one or two carpogonial branches, procarps and their supporting cells filled with protoplasmic contents and stained deeply with haematoxylin and anilin blue; cystocarpic conceptacles slightly convex, (230–) 273–380 μ high, 292–462 μ diam., carpospores arising from the periphery of fusion cell which is often discontinuous in section; spermatangial conceptacles nearly flat on surface, sometimes embedded deeply, 90–230 μ high, 290–460(–700) μ diam., opened by a very narrow orifice, without a spout,

spermatangia globular, 3 μ diam., when detached and set free in conceptacle cavity which is wholly lined with a layer of uninucleate palisade-like cells or spermatangium mother cells.

The genus *Polyporolithon* was established by L. R. Mason in 1953 to comprise four hemiparasitic and mushroom-like species formerly classified in *Lithothamnium*, one from Australia and New Zealand and the rest from the Pacific coast of North America. The writers' specimens from Muroran are referable to the present species as the above description shows. The male plant which has hitherto been unknown to this species is described here for the first time. The spermatangial conceptacles are quite peculiar in structure as shown in Pl. III, Fig. 2. A specimen of *Pachyarthron cretaceum* collected by Mr. Hidehiro Niihama at Shirikishinai which has been deposited in the writers' herbarium bears an epiphyte, a sporangial disk of *Polyporolithon reclinatum*.

The present species is at first a small circular disk attaching laterally on the internode surface of a single articulation of the host by means of a sub-penetrating base (Pl. I, Figs. 1f & 2f; Pl. II, Fig. 2). The disk later becomes irregularly expanded laterally or lengthwise, and more or less recurved, sometimes encircling the host partly or completely (Pl. I, Figs. 1 & 2). In such cases the disk may cover several articulations of the host but it has no attachment to them except the original single one (Pl. I, Fig. 2c–e). In some cases, however, several articulations of the host plant may be firmly adherent to one disk of the present species when they are emdedded in the calcareous bodies of epiphytic animals such as a Bryozoa (Pl. I, Fig. 2a) or *Spirorbis* (Pl. I, Fig. 2f). *Spirorbis* is commonly found attached in groups to the lower concave surface of the disk (Pl. I, Fig. 2d f). Its calcareous tubes sometimes happen to hold some of the host articulations making the attachment of the epiphyte to the host much more stable.

Summary

Polyporolithon reclinatum (Foslie) L. R. Mason, which has hitherto been known from the Pacific coast of North America, is reported herein to occur in Hokkaido. Besides the previously described sporangial and female plants, the male plant was newly observed and illustrated.

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(For further references: see the preceding report III).

(**ERRATA** for the preceding report, III, in *Bull. Fac. Fish., Hokkaido Univ.*, 11 (2): read "Foslie, 1929" for "Foslie, 1919" on p. 37, p. 38 and p. 41).

EXPLANATION OF PLATES

PLATE I

Polyporolithon reclinatum (Foslie) L. R. Mason

Habit of plants growing on *Pachyarthron cretaceum* from Muroran

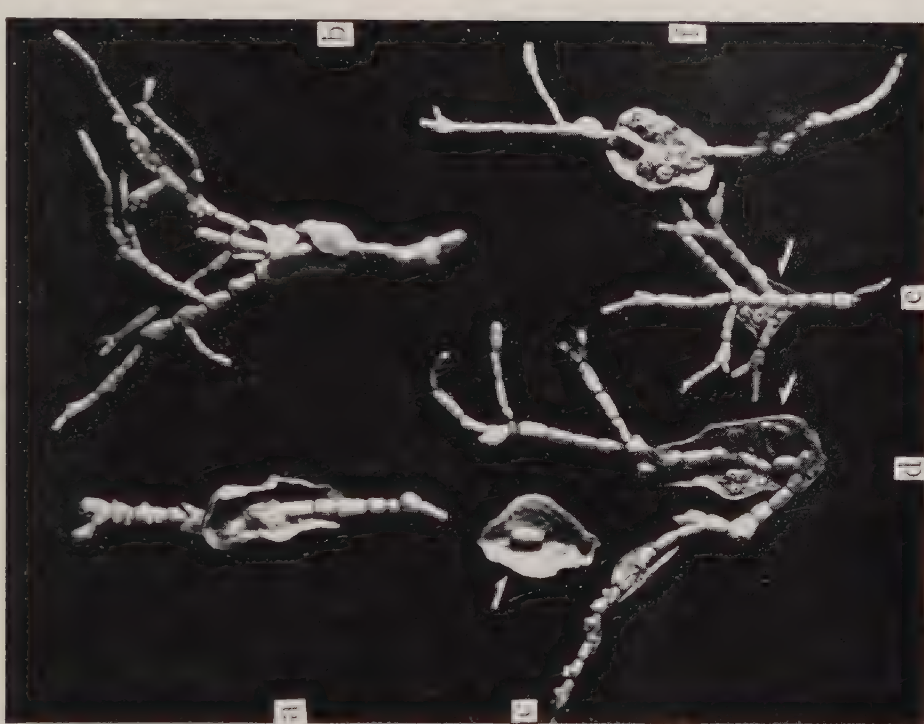
Fig. 1. Viewed from upper surface of thalli

Fig. 2. Viewed from lower surface of the same thalli as shown in Fig. 1

a & b. Thallus encircling host segments completely at least in part

c–e. Thallus in the form of a recurved disk which is firmly attached to a single segment of host indicated by arrow

f. A small young circular disk and an older recurved disk infested with a number of calcareous tubes of *Spirorbis* which hold several segments of the host in a firm attachment to the disk



2



1

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PLATE II

Polyporolithon reclinatum (Foslie) L. R. Mason

Fig. 1. Habit of fertile plant bearing cystocarpic conceptacles ×2.5

Fig. 2. Vertical section through thallus base penetrating into host tissue ×70

[illegible]

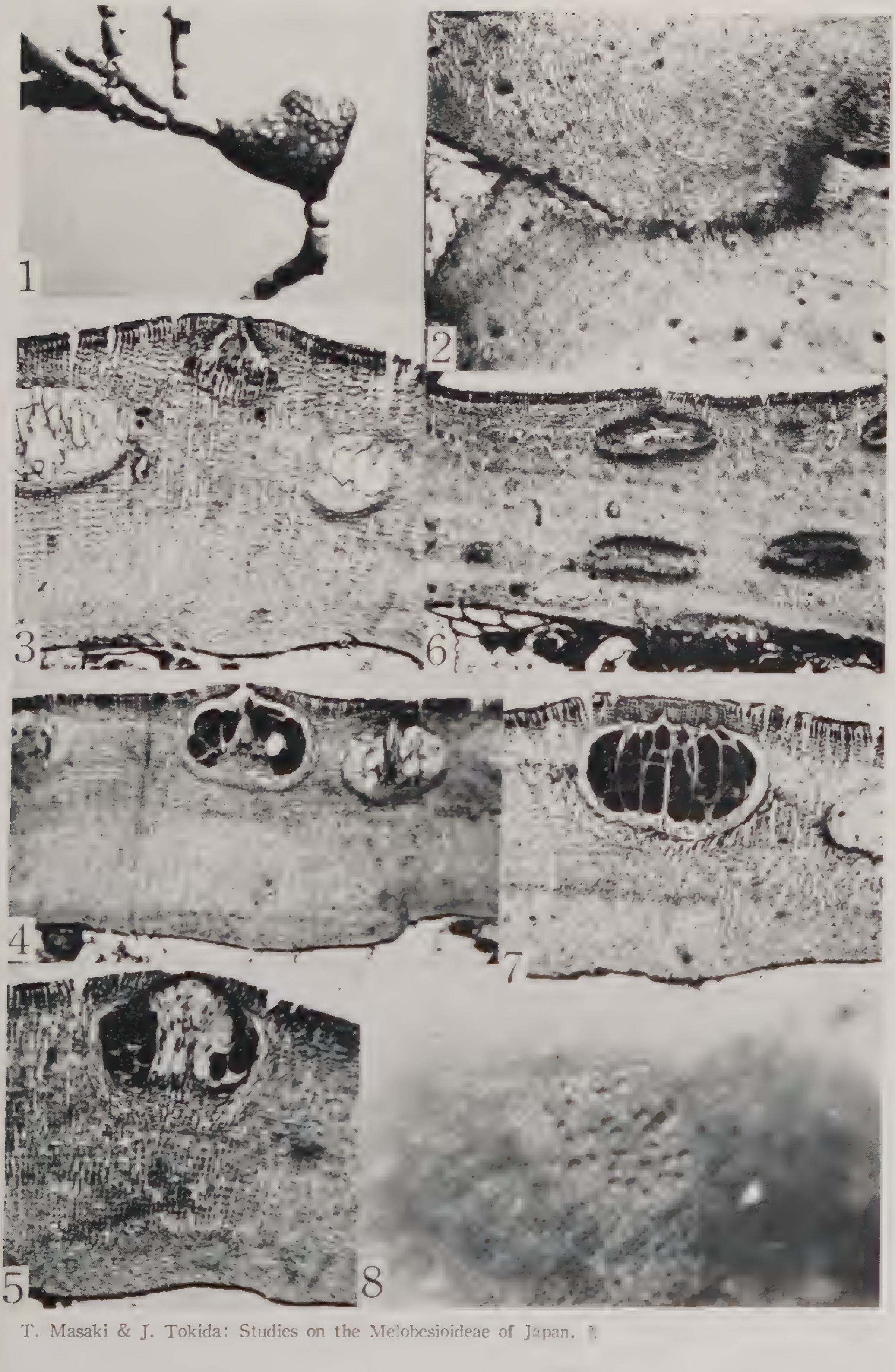
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Fig. 8. Part of surface view of tetrasporangial plant showing pores perforating roof of conceptacle ×70



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PLATE III

Polyporolithon reclinatum (Foslie) L. R. Mason

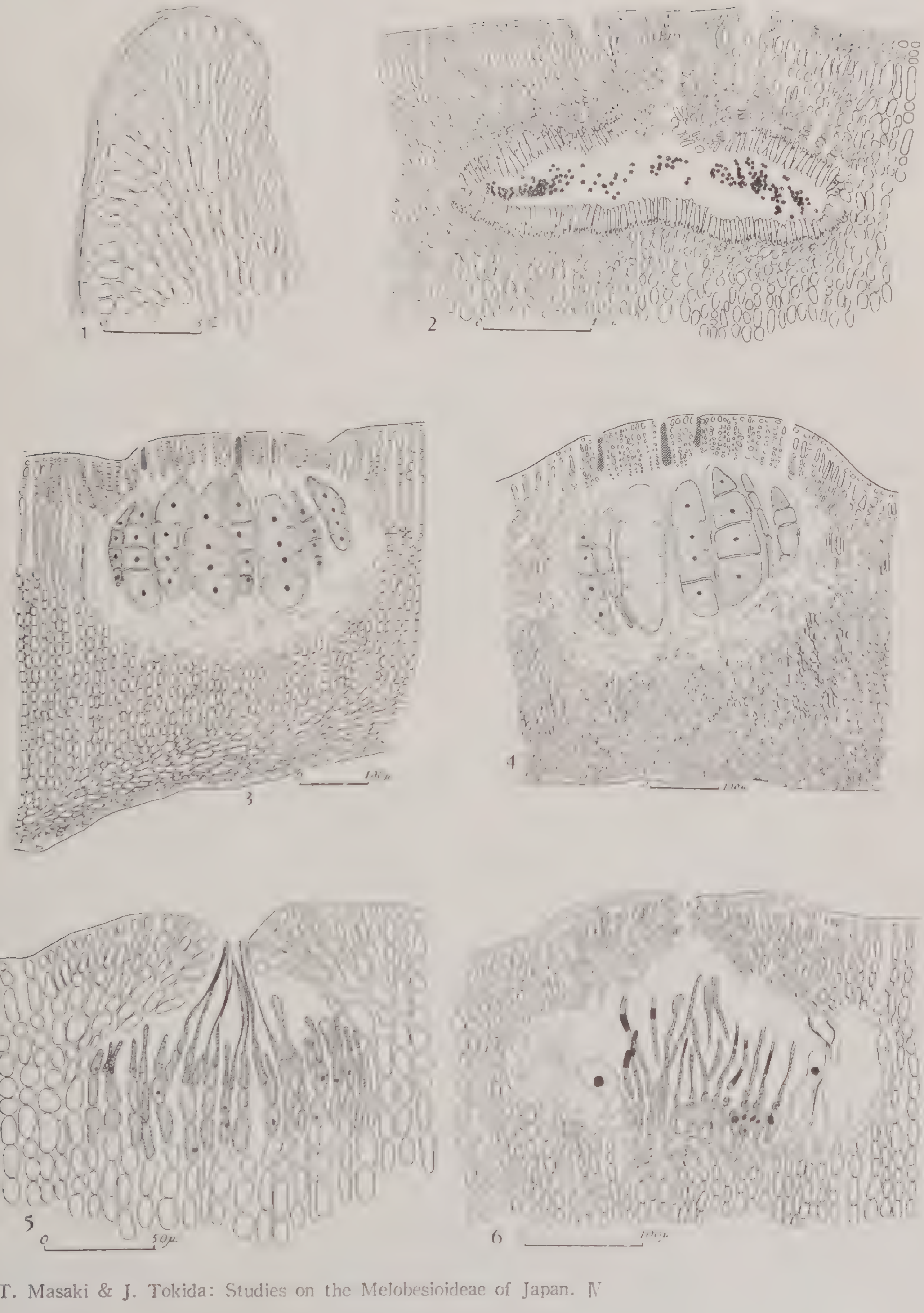
Fig. 1. Vertical section through marginal portion of thallus

Fig. 2. Vertical section through spermatangial conceptacle

Fig. 3 & 4. Vertical section through tetrasporangial conceptacle

Fig. 5. Vertical section through procarpic conceptacle

Fig. 6. Vertical section through young cystocarpic conceptacle

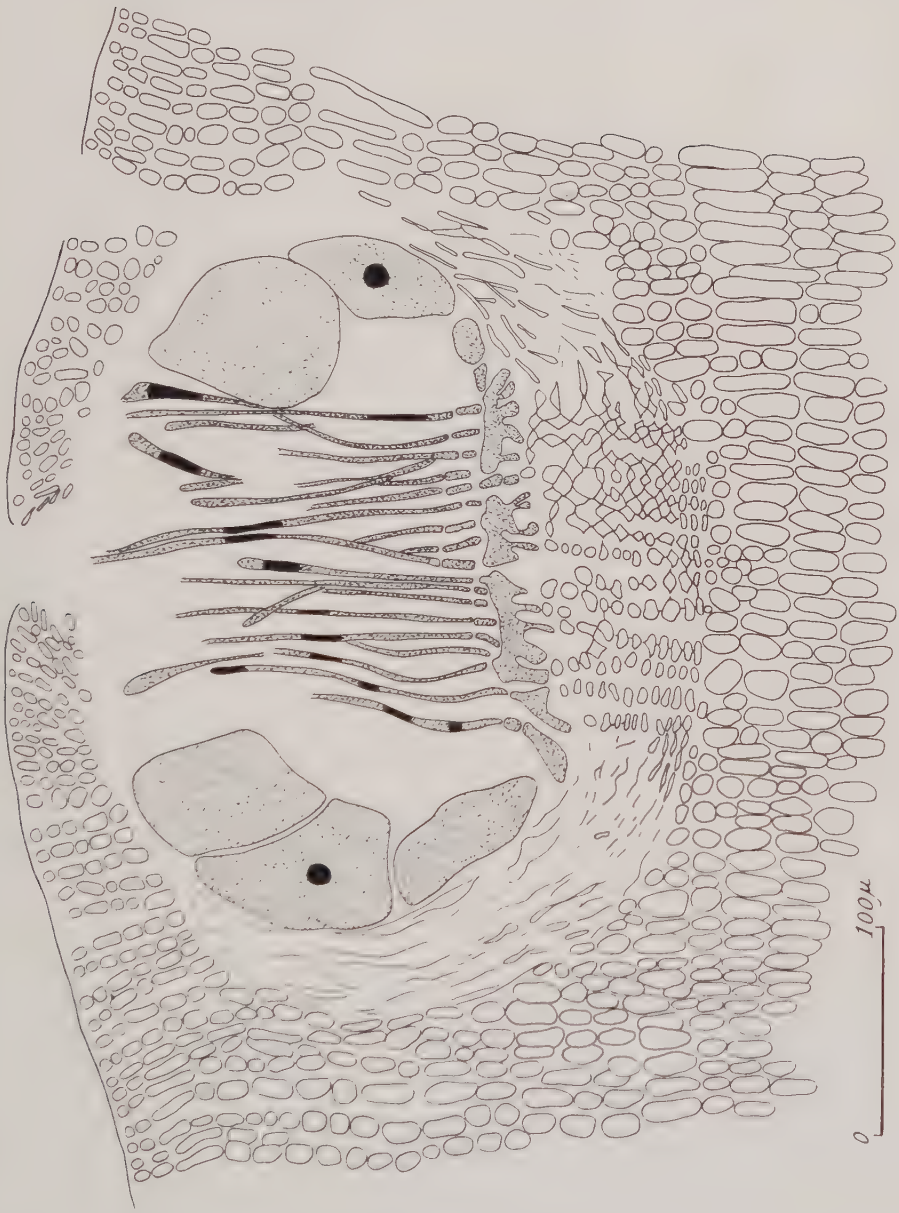


T. Masaki & J. Tokida: Studies on the Melobesioideae of Japan. IV

PLATE IV

Polyporolithon reclinatum (Foslie) L. R. Mason

Vertical section through mature cystocarpic conceptacle .



T. Masaki & J. Tokida : Studies on the Melobesioideae of Japan. IV

PRIMARY RECORD OF *PSENES MACULATUS LÜTKEN*, FROM THE NORTH PACIFIC*,**

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Faculty of Fisheries, Hokkaido University

Hitherto, regarding the species belonging to the genus *Psenes* (Nomeidae) there has been reported only one specimen in each species *Psenes cyanophrys VALENCIENNES* as described by Kamohara ('42) and *Psenes arafrensis GÜNTHER* as described by Tomiyama ('54), from Japanese waters.

One specimen corresponding to *Psenes maculatus LÜTKEN* was collected by the training ship "Oshoro Maru" of Hokkaido Univ., from the North Pacific in 1956. Heretofore, this species was recorded from the comparatively warmer Atlantic only. Accordingly, the author would describe the specimen in this paper.

The specimen was collected with many large jelly-fishes (unidentified), by means of a fish larva net towed in the surface, at 43°44′ N, 154°58′E at 7 p.m. (sunset time) July 16th 1956. (No. L-151)

Here, the author wishes to express heartfelt thanks to the late Prof. S. I. Sato of Fac. of Fish., Hokkaido Univ., under whose guidance and criticism this work has been done. Cordial thanks are offered to Dr. T. Abe of Tōkai Reg. Fish. Res. Lab., who gave facilities for consultation of the literature, and moreover gave him various sorts of advice. Also, he wishes to acknowledge his indebtedness to Capt. T. Fujii, Officers and crew of the "Oshoro Maru" and to research members of the cruise of the year 1956, who rendered valuable help in the collection of the specimen.

Psenes maculatus LÜTKEN

Hanten-hanabirauwo (new Japanese name)

Psenes maculatus: Lütken, 1880, Dansk. Vid. Selsk. Skrift. Kjöbenhavn, 5, ser. 12, 518~521; Collett, 1896, Rés. camp. sci, accom. yacht Albert Isou. Monaco, X, 31~33; Jordan & Evermann, 1896, Bull. U. S. Nat. Mus. (49), 951; Regan, 1902, Ann. Mag. Nat. Hist. 10(7), 125~126; Fowler, 1936, Bull. American Mus. Nat. Hist. 70, 664~665; Miller, 1946, Jour. Wash. Acad. Sci. 36(6), 210.

D. X-I, 22; A. III, 21; P. 21; V. I, 5; Scales in L. L. about 55; Gill rakers 9+15 (left side); Vertebrae 15+18=33; Branchiostegals 5.

Total length 49.9mm; body length 36.1mm. Length of head 2.39 in body length; depth of body 2.17. Length of snout 4.19 in head length; maxillary 2.51; interorbital

* 北海道大学練習船調査報告 No. 16.
** 本研究の一部は、昭和35年度文部省科学研究費（佐藤信一）によつてなされた。

width 2.90; depth of caudal peduncle 3.87. Length of longest dorsal spine 2.60 in head length; longest dorsal ray 2.25; longest pectoral ray 1.31; longest anal ray 2.06; longest ventral ray 1.39.

Body is oval, its highest part in the breast. Tip of snout is round, and two nostrils are posited on it, anterior one is especially clear with large hole. Eye large, diameter of orbit 2.69 in head length. Lateral line is running on the dorsal side of about five scales below the dorsal fin but incomplete at caudal portion. All fins are large, the tips of pectoral and ventral beyond the origin of anal fin. The rays of each fin were observed by X-ray, the first dorsal has 10 spines, the second dorsal 1 spine and 22 rays, the anal 3 spines and 21 rays and the caudal ix+8+7+viii, viz., it has 15 straight rays. Teeth are arranged in irregular line on the upper jaw, and more sharp and longer than those on the lower jaw. Palatine teeth 4 on left side and 3 on right side (Fig. 1).

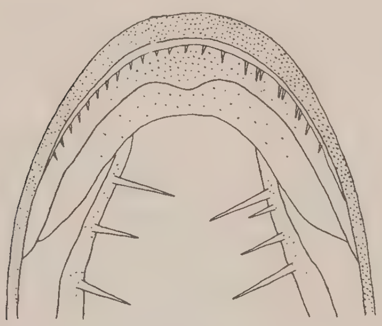


Fig. 1. Inner view of the palate of *Psenes maculatus* Lütken

Color in formalin is nearly light brown with about six dark brown bands, posterior two are extending to the base of dorsal and anal. Anterior half of first dorsal is blackish-brown, and about one-third of the marginal portions of second dorsal and anal are light brown; their other portions are colorless. Caudal light brown in marginal part, however its tips are black. The pectoral is faint in colour and margined with light-black. As for the ventral, about one-third of its middle portion is colorless, and its other portions are black.

On this species, Lütken (1880) made the first record from the Atlantic (39°N～25°4′S, 34°40′W～27°26′W); he clearly indicated that his specimen has teeth on the palatine and vomer. He strongly suspected that his specimen was really the young stage of a *Cubiceps*. Collett (1896) described it from the sea between Newfoundland and the Azores (41°40′37″N, 43°48′10″W) and he emphasized that it was an error in judgment, for Lütken (1880) suspect strongly that his species was really the young stage of a *Cubiceps*. But more recently Miller (1946) mentioned this matter, of Collett's (1896) opinion insisting that his interpretation was wrong, although he seems to have overlooked or ignored the palatine and vomerine dentition. Jordan & Evermann (1896) described the species from the Atlantic (27°49′N, 76°12′W) from the Albatross expedition. They said, according agreeing with Lütken, the specimen was very likely the young of *Psenes capensis* (*Cubiceps capensis* or *gracilis*), but that species has D. X-I,19; A. I,19. Regan (1902) made a revision of Stromateidae. Genus *Psenes* was distinguished from the genera *Nomeus* and *Cubiceps*,

for *Psenes* has an oval body, is toothless in the palate and the ventral fin is below the pectoral fin. This species should be included in genus *Psenes* according to Lütken (1880); he made a synopsis of the species according to the number of dorsal and anal fin rays, and the depth of body etc. Fowler (1936) made a synopsis of this genus from the western Africa quoting from Lütken (1880), Collett (1896) and Regan (1902). Miller (1946) made observation on the four specimens of U. S. Nat. Mus. He offered discussion on this species quoting from Lütken (1880) and Collett (1896), and he raised a question, whether this species should be included in the genus *Psenes*, but he made no observation on the teeth of palate.

From the above-mentioned, in comparison with the known two species of genus *Psenes* from Japanese waters, this species is distinguished from those two species, according to the points noted here. *P. cyanophrys* has higher caudal peduncle (3.00 in B.L.), and a larger number of dorsal and anal fin rays and gill-rakers. *P. arafrensis* has greater depth of body (1.75 in B.L.) and shorter snout (5.00 in H.L.), and a smaller number of lateral scales. Besides, this species is distinguished from *P. arafrensis* of Tomiyama's ('54) specimen being 58mm in total length and lacking palatine and vomerine teeth.

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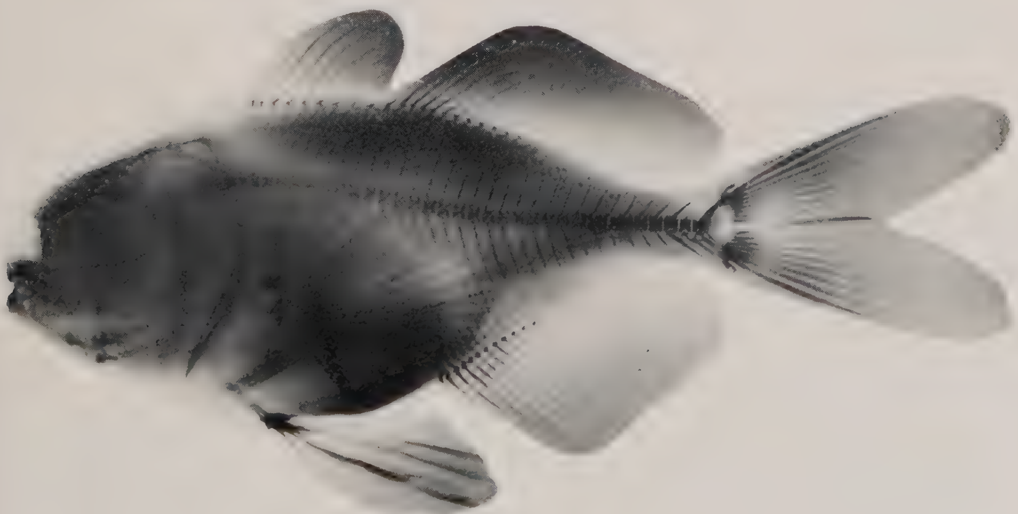
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Fig. 1. *Psenes maculatus* Lütken T. L. 49.9mm (No. L-151)



Fig. 2. Radiograph of the specimen shown in fig. 1



STUDIES ON THE BIOLOGY OF THE SEA URCHIN

IV. Histological Observation of the Food Canal of *Strongylocentrotus intermedius*

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Anatomical and histological descriptions of the food canal of the sea urchin given by Hamann (1887), Chadwick (1900), and Stott (1955), may be incomplete and certain features require confirmation, bacause they have not presented any descriptions concerning the reserved materials and other biochemical elements, which seem necessary in a discussion of the functional aspects of the food canal. Although *Strongylocentrotus intermedius* is a common subtidal species in much of its range along the coast of Hokkaido, no detailed anatomical or histological studies of its food canal appear to have been made.

Consequently, the present report is an account of studies undertaken to clear up some of the existing gaps in knowledge of the histology of the food canal, and to correlate by means of histochemical techniques the structual and functional aspects of the cells comprising the lining epithelium of the food canal.

Before proceeding further, the writer is pleased to record his indebtedness to Prof. T. Tamura of Hokkaido University, for his continued encouragement and guidance. Acknowledgement is also due to Mr. Y. Ogawa and Mr. H. Kimura for the supply of living materials.

Material and Method

For most of the experiments reported here, sea urchins were collected at Sumiyoshi, Hakodate. In a few instances specimens were obtained from Ishiya, along the coast of Volcano Bay, southern Hokkaido. They were adult specimens ranging in size from 40 mm to 70 mm in test diameter.

They were dissected and portions of the food canal were fixed in a fixative for further study. These materials were subsequently processed and imbedded as appropriate for use of the following histological and histochemical techniques.

For general orientation of cell structure, tissues were fixed in Bouin's fixative, imbedded in paraffin, and sectioned at 8 μ. These sections were stained with Heidenhain's iron alum haematoxylin or Delafield haematoxylin.

For demonstration of glycogen and related compounds, fixation was in Gender's Allen Bouin. After preparation of paraffin sections, the periodic acid Schiff (PAS) routine was followed. Control slides expsoed to the action of a buffered solution of saliva were

used for differentiation between glycogen and other PAS-positive substances. For the recognition of mucin and other similar compounds, paraffin sections of tissues fixed in 10% formallin were stained overnight in a very dilute aqueous solution of toluidine blue and dehydrated with 50% alcohol. This routine brings about a metachromatic staining of acidpolysaccharide elements.

For recognition of lipid, tissues fixed in formal-saline, were imbedded in gelatin, and frozen sectioned at 10 μ. Sections were mounted in 1% gelatin solution then coloured with Sudan Ⅲ.

Alkalin phosphatase activity was detected in tissue fixed in 80% alcohol, and carried through the routine of Gomori.

Observation

The food canal of the sea urchin has four parts: pharynx, oesophagus, gut, and rectum. The gut has two loops; the first loop is clockwise, looking from the mouth, and the second doubles back upon the first one. The first loop differs from the second loop in having different nutritive functions and cell contents as pointed out in the following description. Accordingly these loops are indicated in terms of *stomach* and *intestine* respectively in the present paper.

The wall of the food canal is relatively simple; the presence of four main layers of tissue is observed throughout the whole length of the gut. In respect to histology, they consist of an outer epithelium of flat epithelial cells whose distal surface is bathed by coelomic fluid, a layer of definite bands of circular muscle and of longitudinal muscle cells, a layer of connective tissue of varying thickness, and an inner epithelium of a single layer of extremely tall and slender cells. The inner epithelial layer contains several distinct sorts of gland cells described below in detail. The epithelial lining is chiefly responsible for the thickness of the wall. The thickness of the epithelium varies markedly, it may ranged 50 - 200 μ.

The following description is concerned with elucidation of the cellular constituents and their characterization of the lining epithelium of the various parts, in terms of their histological and histochemical details.

Pharynx: The pharyngeal wall is deeply grooved, therefore it may be enlarged and contracted during the passage of food materials. There are also attachments of connective tissue running from the lips to the compasses of Aristotle's lantern. In the mid-pharyngeal region the well-marked grooves in the radial regions are almost exclusively lined with numerous mucous gland cells, and these together with secretory cells with strongly basophilic granules were distributed at random amongst simple epithelial cells (Fig. 1).

Mucous gland cells average about 5 μ in diameter and vary in height from 100 μ to a maximum of about 200 μ, depending upon their location and upon the size of the

animal. Their nuclei are broadly oval shaped, measuring about 3 $\mu \times 5 \mu$, and are approximately situated at the middle third of the cells. The space is filled with granules which stained slightly with haematoxylin. At a variable distance from the base, the cell narrows and finally opens into the lumen of the pharynx at a restricted stoma (Fig. 1). The basophilic granules are comparatively larger and more numerous in the basal portions of the cell: above the nuclear region they become smaller and more sparse.

The material composing these granules reacts vigorously to the Schiff reagent after treatment with buffered saliva (Fig. 2), but does not stain metachromatically with dilute toluidine blue. The cells covering the grooved region consist of granules with strongly positive reaction to the Schiff reagent after salivary digestion, while they do not stain metachromatically with dilute toluidine blue in acid solution. From the above data, it seems a justifiable conclusion that the content of these cells is to be regarded as neutral mucopolysaccharides.

The secretory cells with strongly basophilic granules were observed over the free ends of the epithelium; these cells were usually expanded to a diameter many times that of the neighboring cells. The granules of the cells exhibited PAS-negative reaction but stained strongly with haematoxylin. It may be assumed that these cells discharge fluid from the basophilic granules within them, and that the fluid is mainly responsible for the relatively greater acidity of the pharynx as compared with the rest of the food canal (Fuji, unpublished data).

In non-stained materials, a large number of reddish-purple coloured granulocytes, measuring about 15 μ in size, are notable in the connective tissue layer and the basal region of the inner epithelium. Perhaps they are echinochrome containing amoebocytes, and it is suggested that some respiratory action may be carried out there. Moreover, abundant agranulocytes localize in a similar region. Their size measures about 7 μ in diameter. When they are stained with haematoxylin, their cytoplasm appears as clear and as faint basophilic in character (Fig. 3).

Oesophagus: The oesophagus has well developed circular and longitudinal muscles in its wall, and its inner epithelium is a continuous layer of mucous cells. Two types of the secretory gland cells are responsible for the production of secretion (Fig. 4). One type of the secretory cells possesses the strongly basophilic granules situated from a region near the basement membrane to the free edge of the cell, althouth these granules are larger and more numerous in the apical region of the cell. These granules were strongly stained with haematoxylin, whilst they gave a very faint PAS reaction (Fig. 5). The other type contains numerous granules limited to the apical portion of the cell. In addititión to being PAS-positive, their materials are basophilic but are not stained metachromatically with dilute toluidine blue. In all secretory cells, the nuclei lie at various depths in the epithelium, from near the distal end to the middle of the cells.

Stomach: The inner epithelium of the stomach region is composed of cylindrical cells. These cells are of somewhat variable length (100 – 150 μ) and gave rise to ridges on the inside wall of the stomach. Their nuclei are similar in size and shape to those of the secretory cells in the pharynx, and lie at various depths in the tall cells, from a point near the basal end to the middle of the cells.

The outstanding feature of the secretory cells is their content of secretory granules. These very tiny, yellowish, refringent granules lie in clumps in the slightly expanded region adjacent to the nucleus, but they have not been observed below the nucleus; they may extend in one or many rows completely to the free end of the cell (Fig. 6). These secretory spherules are encountered very frequently in the stomach epithelium alone, and there is never observed even a trace of such materials in the rest of the gut.

Several histochemical techniques were carried out to demonstrate some additional characteristics in various histological components of the food canal. Small deposits of glycogen are distributed in the basal portions of the cells (Fig. 7), especially their occurrence is more numerous in the hinder half of the stomach. After removal by salivary digestion, of all PAS-reactivity attributable to glycogen, several sites retain strongly positive reactions. These sites are limited to the connective tissue basement and the surface region of the inner epithelium of the stomach wall (Fig. 8). In addition to showing the above reaction, these sites are faintly basophilic but do not stain metachromatically with toluidine blue.

In a normal, well-fed animal these cells contain some deposits of lipids, which stain in pink with Sudan III. The lipids forming moderate-size droplets, are distributed in the basal portions and are usually lacking from the distal quarter of the cells (Fig. 9).

Alkalin phosphatase activity appears to be largely limited to the free edge of the inner epithelial region, but it is weak or lacking in the deeper portions of the epithelium (Fig. 10). This enzymatic activity is stronger in the fore half region than in the hinder one.

Larger eosinophilic granulocytes, measuring about 10 – 15 $\mu \times$ 15 – 25 μ, are sometimes found to be imbedded within the inner epithelium. Such granulocytes show yellowish-coloured in non-stained materials. The absorption of ingested material was tested by feeding the animals on starch solution containing carbon particles (ca. under 5 μ in size). Fed individuals were left for definite periods after which the food canal was fixed with Bouin's fluid. Normal paraffin imbedded sections were employed. In the stomach region, within the epithelium one or more carbon particles lay inside the granulocytes, which were distributed heavily near the free edges of the epithelium (Figs. 11 & 12). However, transverse sections from all parts of the pharynx and oesophagus of specimens fed on carbon particles did not exhibit the presence of any granulocytes. The intestine and rectum of animals fed with carbon particles showed absence of the granulocytes from the epithelial layer.

Intestine and Rectum: The intestine differed from the stomach in its relatively thinner walls, and lack of yellowish, refringent granules and of any basophilic granules.

Detailed examination shows the intestine to have an inner epithelium composed of cylindrical cells measuring approximately 70 – 150 μ in height (Fig. 13). The cytoplasm was clear, homogeneous and stained faintly with haematoxylin. The nuclei lie at various depths in the epithelium.

Histologically the rectum was similar to the intestine except that the inner epithelium was more thick, measuring about 30 – 70 μ in height of the cells (Fig. 14).

In the fore half of the intestine, glycogen and lipid deposits are shown in the basal region of the inner epithelium, while alkalin phosphatase activity is limited at the free end of the epithelium (Fig. 15). Glycogen and lipid deposits are never found in the rectum. Alkalin phosphatase, however, still appears in the marginal region of the inner epithelium, although its activity is weak.

Discussion

The histology , morphology and functioning of the food canal in the sea urchin has been the subject of several investigations varying in accuracy and in the interpretation of the results observed. In his book, "Vergleichende chemische Physiologie der niederen Tiere," Fürth (1903) gave a rough description concerning the food canal of the sea urchin. The account of Lasker & Giese (1954) of the nutrition of the purple sea urchin, *Strongylocentrotus purpuratus*, includes the food canal, but there is little communication of histological information. Concerning the cell population of the epithelium there is no analysis. Recently, Stott (1955) published in some detail the structure and function of the food canal and associated haemal canals in *Echinus esculentus*. Stott's findings on the secretory cells in the pharyngeal epithelium agree with the present writer's findings on *S. intermedius* in so far as the presence of two kinds of secretory cells is concerned. However, there will be noticed that the writer's findings differ from Stott's findings in some morphological descriptions of these secretory cells. In *Echinus esculentus*, the basophilic granules and mucous granules in the secretory cells are localized uniformly over the whole of the cells, from the figures presented by Stott, but the location of these cells was not stated, while in *S. intermedius* as shown in the previous section, the mucous gland cells contain slightly basophilic granules, which are larger and more numerous in the basal portion than above the nuclear region of the cell. Moreover, secretory cells with strong basophilic granules are observed over the free ends of the epithelium and they are located dominantly near the grooved region. From Stott's (1955) description and figures the wall of the oesophagus is composed simply of a continuous layer of tall, narrow, cylindrical mucous secreting cells. Using certain histological and histochemical techniques, the present author observed that two types of secretory cells are responsible for the

production of this secretion; one forms a neutral mucopolysaccharide only, whilst the other contains remarkably basophilic granules. From the above findings, the author considers that the latter produce an acid secretion which may be responsible for the relatively greater acidity (ca. pH 6.2) of the oesophagus, like that in the pharyngeal region.

The inner epithelium of the stomach consists of long columular cells which contain chains of tiny yellowish granules. These granules are distributed in the stomach epithelium proper. Digestive enzymes of the stomach extract predominate in their activity over those in the rest of the food canal (Fuji, unpublished data). From superficial morphology of the gut, without any observation of enzymatic activity, Delanuay (1931) has suggested that the first half of the gut itself possesses an active digestive function. From above information, it seems probable that these granules located in the stomach epithelium are discharged though the free surface and break down presumably to provide the extracellular enzymes of the lumen.

Accoding to Hilts & Giese (1949), chemical analysis demonstrated an appearence of some amounts of glycogen in various tissues of the sea urchin, but most appears in the stomach. Lasker & Giese (1954) on the basis of chemical analysis pointed out that a considerable store of glycogen exists in the food canal. Glycogen and lipid deposits are histochemically demonstrable in the hinder half of the stomach and in the fore half of the intestine of the sea urchin used in the present investigation. The conclusion that these materials are food reserves rests upon the evidence that it is possible to demonstrate a detectable decrease after five week's starvation. Therefore, the above opinions concerning the storage matter derived from chemical analysis are also justfied by the results obtained from the present investigation. From the appearence of alkalin phosphatase activity at the free end of the gut epithelium, it is strongly suggested that the gut possesses an absorptive function. Stott (1955) and other previous workers did not demonstrate any appearence of some biochemical elements in the food canal by reliable techniques. It seems necessary to discuss the functional aspects of the food canal.

In the case of experimental feeding with carbon granules, the inner epithelium of the stomach contains more numerous granulocytes in comparison with that of an animal obtained from the natural habitats. The results of the feeding experiments with carbon granules suggest that absorption of carbon takes the form of migration of the granulocytes loaded with carbon through the stomach wall into the haemal canals. Kindred (1924, 1926) observed that certain granulocytes of the sea urchin, *Arbacia*, liberate their protein or lipid inclusions throughout the organism and that they take up nutrient matter from the digestive system and transform it into the cytoplasmic granules. In consideration of the above information, it may be indubitable that amoebocytes in the stomach epithelium participate in nutrition to a greater or lesser degree by ingesting particles and absorbing dissolved nutrient.

The present observation concerned with the histological and histochemical complement of the food canal may be summarized as follows. The oesophagus has well-developed circular and longitudinal muscles in its wall; its inner epithelium is a continuous layer of mucous cells. Consequently, it is assumed that food materials ingested are propelled to the stomach along the oesophageal region by a powerful peristalsis of those circular and longitudinal muscles. Forthermore, the mucus secreted from its inner epithelium may assist the propulsion of the food by providing lubrication. Proteins and carbohydrates in the ingested material are digested and absorbed in the stomach region. Some of the nutritive substances are elaborated into reserves of polysaccharide and lipid nature, while others are transported to the other organs for storage or for use. The intestine and the rectum act as absorptive organs and a conduction tube for the undigested food materials.

Few investigations have been made of the haemal system which may serve as an avenue for nutritive substances between the gut and other organs (Stott, 1955), associated with the coelomic fluid which has been suggested as a nutritive pathway (Greenfield et al., 1958; Giese et al., 1959). The physiology of the haemal system and of the coelomic fluid is quite unknown from the viewpoint of a possible nutritive role.

Summary

Histological and histochemical observations in different parts of the food canal have been carried out in *Strongylocentrotus intermedius*. The results may be summarized as follows:

(1) The wall of the food canal is composed of an outer peritoneum; layers of circular and longitudinal muscle; a layer of connective tissue of varying thickness; and an inner epithelium generally composed of very tall, slender cells.

(2) Two kinds of secretory cells have been recognized in the pharyngeal and oesophageal wall; one produces a mucoid secretion and the other an acid secretion. Numerous echinochrom-containing amoebocytes and agranulocytes are distributed in the connective tissue layer and the basal region of the pharyngeal inner epithelium.

(3) The stomach is lined with an inner epithelium containing of yellowish granules, which are discharged from its surface to provide extracellular enzymes. Large eosinophilic granulocytes, which are assumed to participate in digestion by ingesting particles and absorbing dissolved nutrient substances, are sometimes found to be imbedded within the stomach inner epithelium. The intestine and the rectum, however, have never been found to contain even a trace of any secretory granules nor of any amoebocytes in the inner epithelium.

(4) Lipids and glycogen, which are reserve materials, appear to be deposited in the hinder half of the stomach and the fore half of the intestine. They are very strong at the deeper portion of the inner epithelium. Starvation for five weeks results in an

appreciable decrease of the reserves.

(5) Alkalin phosphatase activity is shown at the free border of the inner epithelium in the stomach, intestine, and rectum.

(6) It may be certainly concluded that the stomach functions in digestion of food, in absorption of the products of digestion, and that the intestine and the rectum act as absorptive organs and a conducting tube for the undigested food materials.

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EXPLANATION OF PLATES

Photomicrographs of transverse sections through the various parts of the food canal of *Strongylocentrotus intermedius*

PLATE I

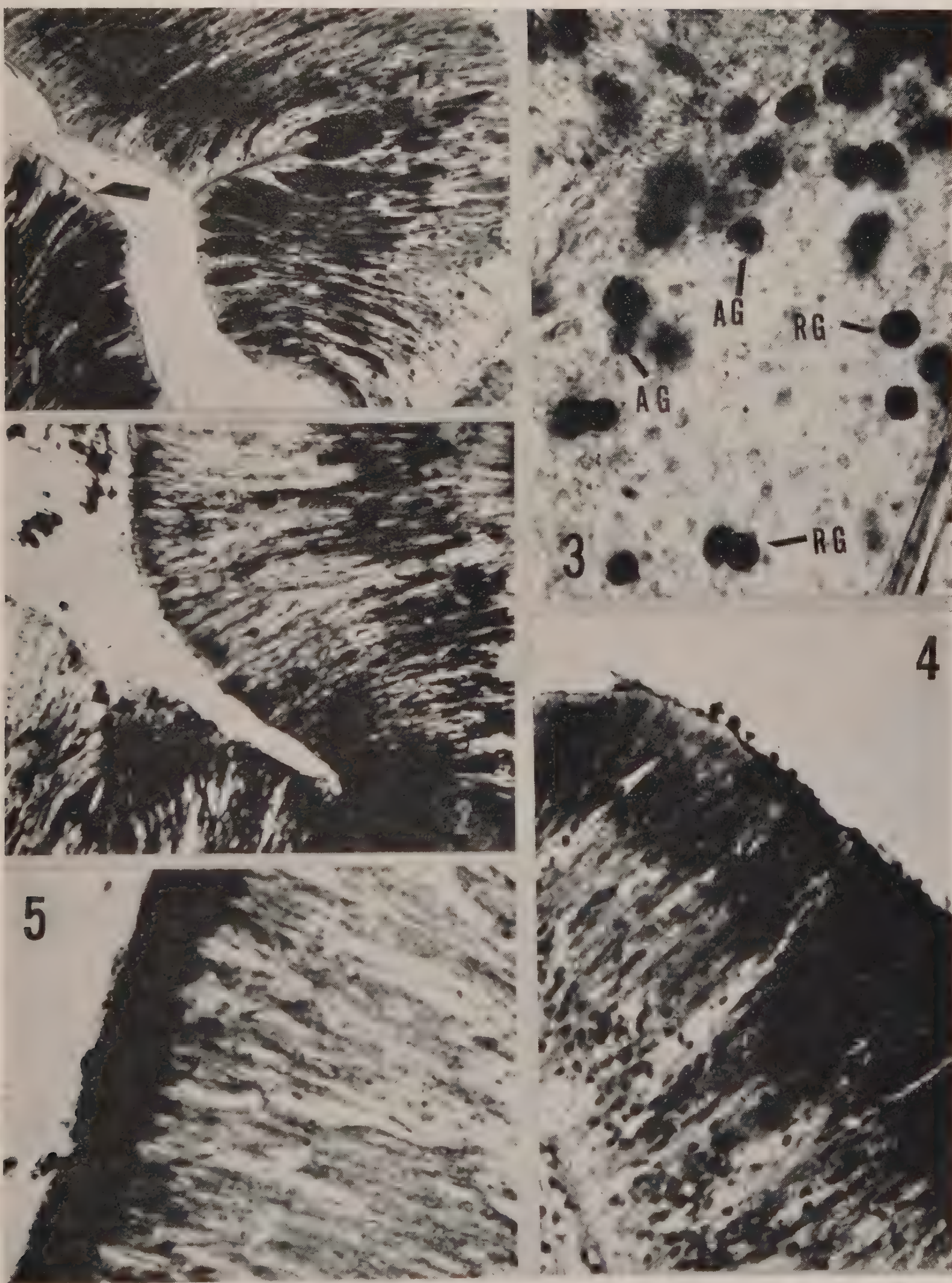
Fig. 1. Mid-pharyngeal region showing the two kinds of secretory cells. A mucous stoma is indicated by the arrow. Bouin-Haematoxylin. ×250

Fig. 2. Part of the same region. Note basal concentration of PAS-positive materials, which have not been removed by salivary digestion. ×250

Fig. 3. Amoebocytes in the basement and connective tissue layer of pharynx. RG, Red-coloured granulocytes containing echinochrome: AG, Agranulocytes. Peritoneum at lower right. Bouin-Haematoxylin. ×500

Fig. 4. Cross section through the oesophagus. Peritoneum at lower left, lumen off upper right. Bouin-Haematoxylin. ×500

Fig. 5. Same region showing heavy concentration of PAS-positive materials, after salivary digestion. PAS-technique. ×500



A. FUJI: Studies on the biology of the sea urchin IV

PLATE II

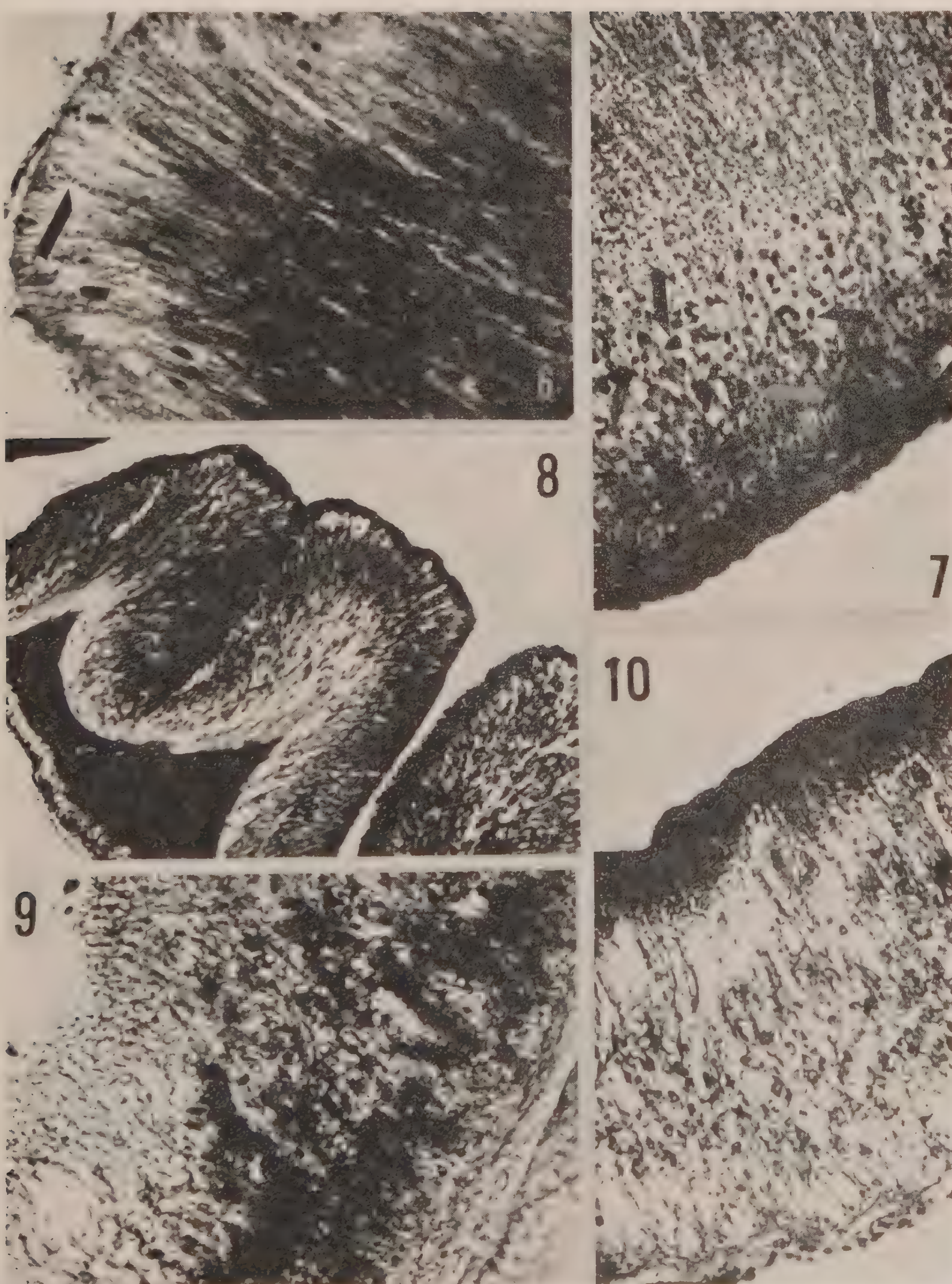
Fig. 6. Cross section through the stomach. The arrow indicates secretory granules extended in one or many rows completely to the free end (left side) of the cell. Bouin-Haematoxylin. ×500

Fig. 7. Basal portion of stomach cells showing glycogen deposits. Arrows indicate small deposits of glycogen. Peritoneum at lower right. PAS-technique. ×500

Fig. 8. Cross section through the stomach region, showing PAS-positive material after salivary digestion. Peritoneum at lower left, lumen of stomach at upper right. PAS-technique. ×370

Fig. 9. Basal region of stomach. Lipid deposits are black. Peritoneum at lower right. Formal-salin: Frozen section coloured with Sudan III. ×500

Fig. 10. Cross section through stomach showing alkalin phosphatase activity. Note concentration of activity at apical region (upper left) of the inner epithelium. Gomori technique. ×370



A. FUJI: Studies on the biology of the sea urchin IV

PLATE Ⅲ

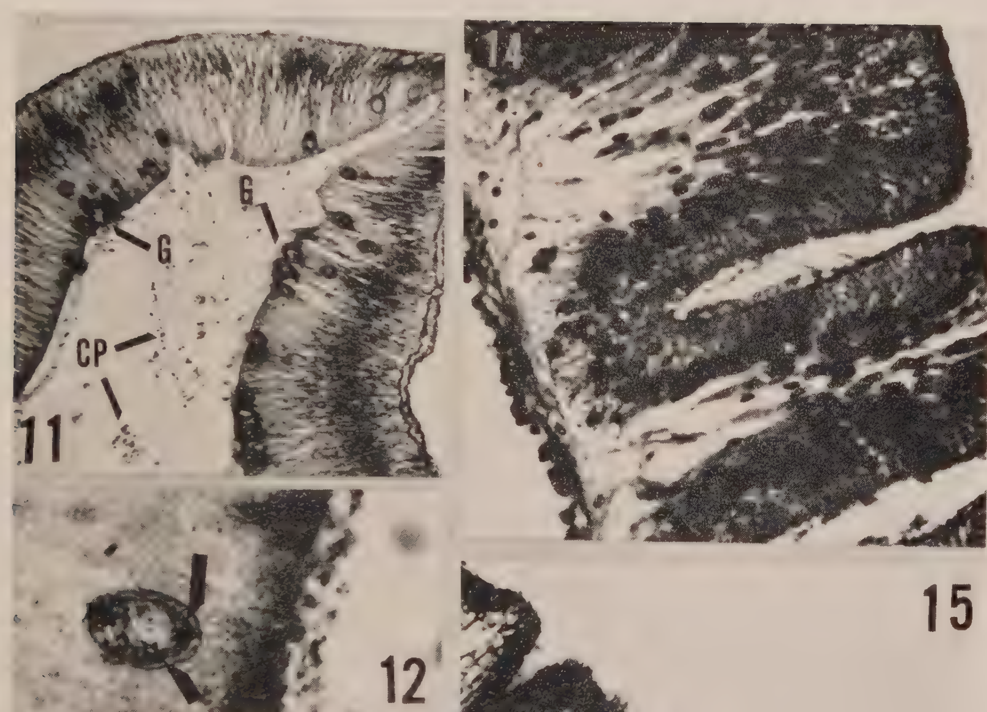
Fig. 11. Stomach region showing heavy distribution of large granulocytes. G, Granulocytes; CP, Carbon particle in the lumen of stomach. Bouin-Haematoxylin. ×120

Fig. 12. Granulocyte imbedded within the inner epithelium of stomach. Arrows indicate carbon particles in the granulocyte. Bouin-Haematoxylin. ×680

Fig. 13. Cross section through intestine. Lumen at upper left. Bouin-Haematoxylin. ×500

Fig. 14. Cross section through rectum. Peritoneum at lower left. Bouin-Haematoxylin. ×500

Fig. 15. Intestinal region, showing alkalin phosphatase activity. Localization of alkalin phosphatase is the black area at apical margin (upper right). Gomori technique. ×370



A. FUJI: Studies on the biology of the sea urchin I\

CHEMICAL STUDIES ON THE MEAT OF "SUKETODARA" (*THERAGRA CHALCOGRAMMA*)-II

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III. CHEMICAL PROPERTIES OF "SUKETODARA" MEAT PROTEIN

4. The change in the amount of proteins dissolved out during bleaching of the "Suketodara" meat in water or 0.6 Mol NaCl solution

When the "Suketodara" meat is soaked in water or NaCl solution, the kind of protein dissolved out from the meat is different as described in the previous experiment[1]. The amount of the protein dissolved in soaking water during the processing of fish paste is considered to influence the quality of the fish paste products.

Here, the authors have examined the change of the dissolved amounts of proteins from the "Suketodara" meat soaked in water or 0.6 Mol NaCl solution for various length of time by estimating the amount of nitrogen dissolved out.

(1) Experimental method

Fresh "Suketodara" was filleted, the meat was removed from the skin and homogenized. Each 5 g of the homogenized meat was put into bottles containing each 50 cc of distilled water or 0.6 Mol NaCl solution and each bottle was shaken and stored. The storing time was 10, 30, 60, 120 minutes and 19, 24 hours at room temperature (at 15°C). After each definite storing time, each bottle was shaken and each content was centrifuged. The total amount of nitrogen in the upper transparent liquor of each content was determined.

The ratio of the dissolved amount of nitrogen to the total amount of nitrogen in the raw meat was calculated as the solubility (%). Also, the intensity of flow birefringence of the upper clear liquor of each content was examined.

Table 15. Changes in the solubility of "Suketodara" (*Theragra chalcogramma*) meat with water and flow birefringence, during the soaking in water for various times

Soaking time	Solubility (% in Total-N)	S.B.
10 mins.	28.13	—
30 〃	28.36	—
60 〃	28.37	—
120 〃	29.29	—
19 hrs.	30.44	—
24 〃	30.53	—

(2) Results

Results are shown in Tables 15 and 16.

As seen in Table 15, the solubility with water increased with the increase of the soaking time; after 19 hours the solubility reached constancy about 30 %. Each extracted solution did not show flow birefringence.

As seen in Table 16, the solu-

Table 16. Changes in the solubility of "Suketodara" (*Theragra chalcogramma*) meat with 0.6 Mol NaCl solution and flow birefringence, during soaking in NaCl solution for various times

Soaking time	Solubility (% in Total-N)	S.B.
10 mins.	—	—
30 〃	44.28	—
60 〃	47.73	—
120 〃	47.27	—
19 hrs.	48.11	—
24 〃	48.20	—

bility with 0.6 Mol NaCl solution increased becoming larger than that with water at initial storing time.

The solubility reached constancy after 19 hours' storage, about 48 %.

No extraction showed flow birefringence as was also true in the previous experiment.

From the results obtained, it was clear that solubility with water or 0.6 Mol NaCl solution increases with the prolongation of the soaking time. But the increase of solubility became almost constant after soaking of 30 minutes. If the time of soaking is longer than 30 minutes, the yield amount of fish paste becomes less. Therefore, when the raw material of fish paste is soaked in water or NaCl solution as a bleaching (soaking) procedure, the soaking of "Suketodara" meat should be less than about 30 minutes.

5. Swelling of the "Suketodara" meat

Inasmuch as the swelling of protein is considered to be one of the phenomena of lyotropic hydration, the authors have examined the phenomenon of swelling of the "Suketodara" meat immersed in various salt solutions and the relation among the degree of swelling, the kind of salts and the pH value of the immersion solution, similarly as was done in the case of the meat of sea-cucumber (*Stichopus japonicus*)[2].

(1) The relation between the degrees of swelling and the concentrations of various kinds of salt solutions

In the presence of salts, according to Migita[3], swelling of meat has an intimate relation to the hydration of the structural protein, in low concentration (0.05 ~ 0.1 Mol) electro-chemical adsorption of salt ions on protein molecules is carried on, and in medium concentration (0.2 ~1.0 Mol) the salt will be adsorbed surface-chemically by protein molecules. At any rate, hydration is accelerated in the range of concentrations as above stated.

The present authors have observed swelling of the "Suketodara" meat soaked in various concentrations of several kinds of salt solutions.

1) Experimental method

Fresh "Suketodara" was filleted; blocks of the meat (2~3 g) were taken, and accurately weighed.

Each block was immersed in 50 cc of the following various salt solutions at room

temperature for about 24 hours. After taking up from immersion liquid, moistened water on the surface of the blocks was wiped off with a filter paper, and the weight of each block was measured. The ratio of the weight of the block meat after immersion (W) to the weight of the block meat before immersion (W_0) was calculated as the degree of swelling (S) of the sample meat ($S=W/W_0$).

Salt solutions employed were NaCl, KCl, $MgCl_2$, K_2SO_4, KCNS, $NaNO_3$, $CH_3COONa \cdot 3H_2O$, $C_3H_4OH(COONa)_3$, NH_4Cl, Na_2CO_3 solutions of 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 and 2.0 Mol and $Na_2B_4O_7 \cdot 10H_2O$ or $Na_3PO_4 \cdot 12H_2O$ solutions of 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 Mol. As contrast, distilled water was also employed.

(2) **Results**

Results obtained are shown in Figs. 12~14.

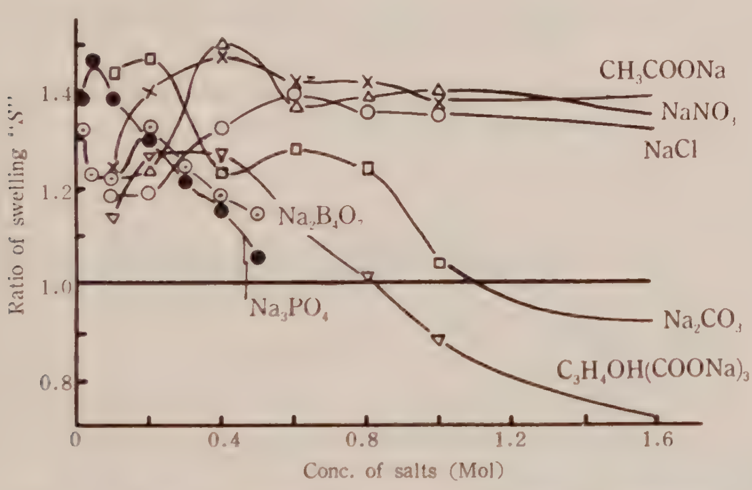


Fig. 12. Degree of swelling of "Suketodara" (*Theragra chalcogramma*) meat in the case of varying anions upon Na^+

As seen in Fig. 12, concerning the influence of varying anions upon Na^+, in monovalent salts, such as NaCl, $NaNO_3$ and CH_3COONa, the degree of swelling of the block meat shows maximum at near 0.4 ~ 0.6 Mol concentrations. In bivalent salts solutions, such as Na_2CO_3, the maximum was shown at near 0.2 Mol. Therefore, the degree of swelling in the lower concentrations was influenced by the ionic valency of Na^+. Comparing organic acid solutions with inorganic acid solutions, in the same ionic valency, for example in $C_3H_4OH(COONa)_3$ and Na_3PO_4, the degree of swelling in the former solution was larger than that in the latter. This is perhaps due to the difference of the dissociating ability between the two solutions. In the concentration of the solutions at above 0.8~1.0 Mol, when the block meat was immersed in the monovalent salt solution, the degree of swelling was almost constant. On the contrary, in the same range of concentrations above stated, the degree of swelling of the block meat immersed in the bivalent salt solution

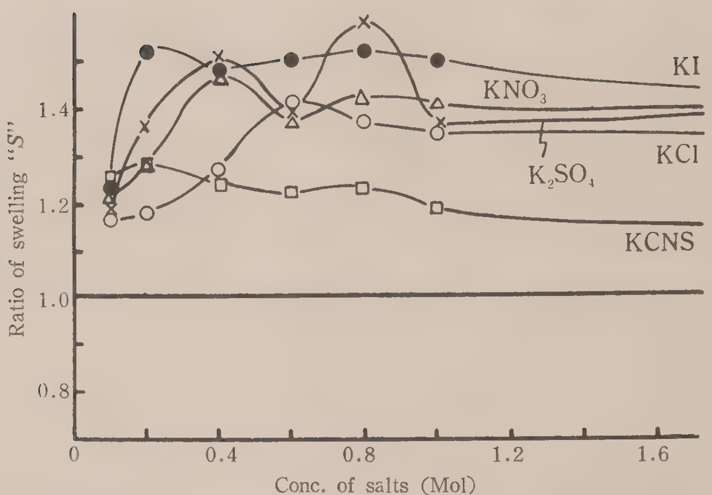


Fig. 13. Degree of swelling of "Suketodara" (*Theragra chalcogramma*) meat in the case of varying anions upon K^+

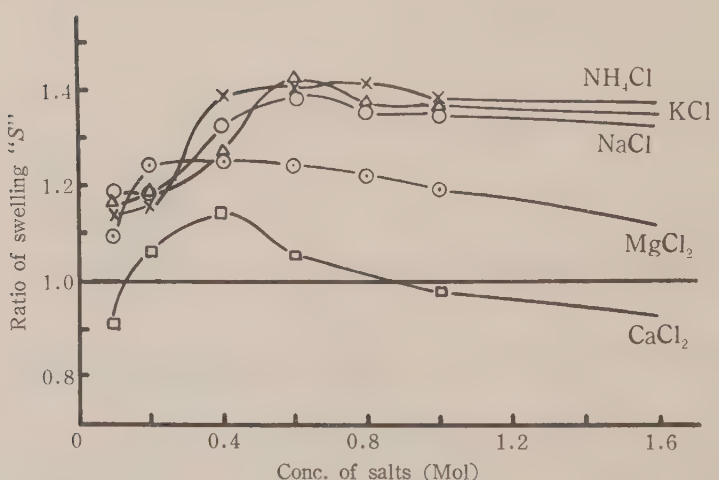


Fig. 14. Degree of swelling of "Suketodara" (*Theragra chalcogramma*) meat in the case of varying cations upon Cl^-

decreased. When the block meat was immersed in Na_3PO_4 or $Na_2B_4O_7$ solutions the degree of swelling showed the maximum at 0.05 Mol or 0.2 Mol respectively.

As seen in Fig. 13, concerning the influence of varying anions upon K^+, the degree of swelling in the salt solutions differs slightly with the kinds of salts. the large degrees of swelling were shown at 0.4 ~ 0.8 Mol. KCNS is different remarkably from other salts, showing the maximum at 0.2 Mol, and its degree of swelling is considered to be non-dependent on the concentration of that salt solution. In the medium concentration of 1 ~ 2 Mol, the solutions of KI, KNO_3, KCl and K_2SO_4 showed the similar degrees of swelling.

As seen in Fig. 14, concerning the influence of varying cations upon Cl^-, in monovalent salt solutions, the degree of swelling shows the maximum at near 0.6 Mol and in bivalent salt solutions the swelling shows the maximum at near 0.4 Mol. In this case, in the lower concentration the degree of swelling depends only slightly on the concentration of the solution. In bivalent salts solutions, such as $MgCl_2$, $CaCl_2$, the degree of swelling shows maximum at near 0.4 Mol, with decrease accompanying increase of the concentration. There is a remarkable difference between the degrees of swelling in monovalent and bivalent salts solution.

From the results described above, the degree of swelling of the "Suketodara" meat varies with differences in the concentrations of the various salt solutions into which it is immersed.

The results obtained from experimental observations on this point are summarized as follows: Below 0.6 Mol of the concentration of salt solutions, the degree of swelling is proportionate to the ion concentration of salts. Above the concentration which shows the maximum degree of swelling, almost all the kinds of salt solutions show decrease of the swelling, especially the degree of swelling in a bivalent salt solution decreased more remarkably than that in a monovalent salt solution. The reason why the degree of swelling decreases above the maximum degree may be due to the fact that until the maximum the meat protein shows swelling because of adsorption of ions, but above the maximum degree, the absorbed water and also a part of bound water in the meat will decrease according to the osmotic dehydrating phenomenon.

That is to say, the equivalent point between the absorption of water by salts and the dehydration is shown as the maximum point of degree of swelling.

(2) The influence of the hydrogen ion concentration upon the degree of swelling of the "Suketodara" meat

As one of the factors influencing the degree of swelling of fish meat, the pH of the solution in which it is soaked has been frequently studied by many investigators[1,6,7,8,9].

The present authors have also studied the influence of pH upon the swelling of the meat of "Suketodara" (*Theragra chalcogramma*).

1) Experimental method

The experimental method and the detection of the degree of swelling were carried on in the same manner as that described in the previous Article.

pH of the immersing solution was adjusted by varying the proportion of the mixture of N/25 HCl and N/25 NaOH covering the range of pH 1 to 13.

2) Experimental result

Results obtained are shown in Fig. 15.

As seen in Fig. 15, the degree of the swelling of the meat is small in the solutions

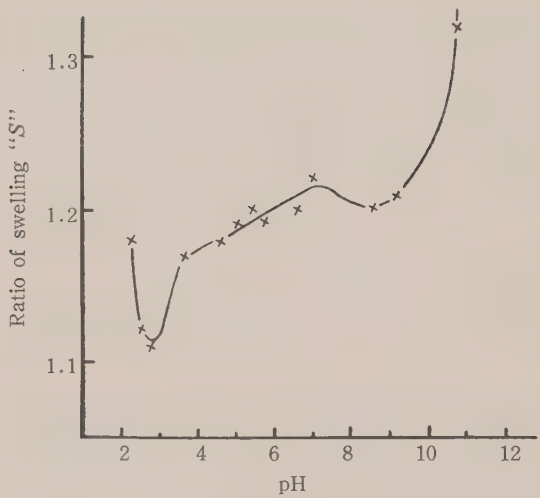


Fig. 15. Degree of swelling of the "Suketodara" (*Theragra chalcogramma*) meat in various pH solutions

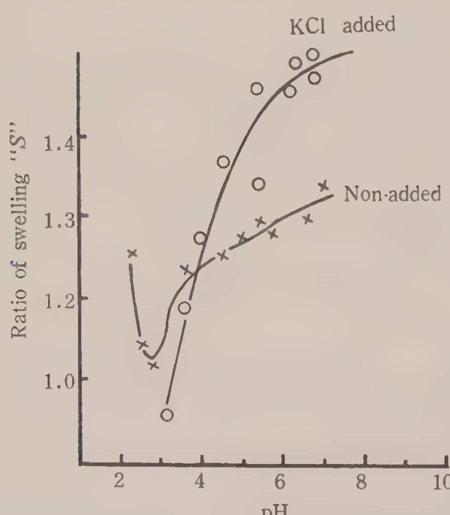


Fig. 16. Degree of swelling of the "Suketodara" (*Theragra chalcogramma*) meat in 0.6 Mol KCl solution having various pH values

on acidic side, but becomes larger from pH 5.0 to the alkaline side.

Generally the swelling or the hydration of protein is minimum in degree at the isoelectric point (pH 5.2~5.4).

Tarr[4], Noguchi[5], Okada[6], and Takahashi[8] have studied the same subject using fish meat or fish skin.

They have observed that the degree of the swelling of fish meat shows minimum value at the isoelectric point (pH 4 ~ 5) and maximum value on alkaline side (pH 10 ~ 12) or acidic side (pH 2 ~ 3).

(3) The swelling of the "Suketodara" meat soaked in various pH solutions in the case of definite concentration of KCl

1) Experimental method

Next, to the immersing solutions having various pH values, KCl was added to make concentration of 0.6 Mol. The swelling of meat blocks soaked in those solutions was estimated similarly to procedure described in the previous Article.

2) Experimental result

Results obtained are shown in Fig. 16.

As seen in Fig. 16, the existence of a neutral salt such as KCl increases the degree of swelling in the various pH solutions. The increase was remarkable until pH 5.2. Above pH 5.2, the swelling became apparently constant. In the acidic side the swelling was inhibited by the presence of KCl. Those results agree with those of Tarr[4] on halibut meat and those of Fujii[7] on Atka mackerel meat.

General speaking, the swelling of the "Suketodara" meat was remarkably large in

the alkaline side above pH 5.2 and small in the acidic side below pH 5.2.

6. Dehydration and coagulation of the "Suketodara" meat dy heating

It is important to understand the phenomena of coagulation and ratio of dehydration by heating in order to determine the heating conditions during the processing of fish paste ("Kamaboko").

Here, the authors discuss their attempt to estimate the ratios of absorption of water or of dehydration of the "Suketodara" meat heated in water at various temperatures, and heat coagulation of proteins which were extracted by water or NaCl solution.

(1) Absorption of water or dehydration

When fish meat is soaked in water at comparatively lower temperatures, the meat absorbs water, but when the meat is heated in hot water dehydration occurs.

1) Experimental method

Fresh "Suketodara" was filleted. The skin was removed from the meat. Blocks of the meat, 1×1×1 cm (2～3 g), were prepared, and weighed. Some blocks were put into a beaker containing 50 cc of water. The temperature of the water was brought up slowly to each pre-determined definite temperature from 20° to 90°C by heating. After the blocks were heated at each definite temperature for 10 minutes, they were taken out from the beaker, and cooled. The water attached to the surface of the blocks was wiped off with filter paper. The blocks were then weighed. The difference between the weight of the block before and after the immersing in heated water was obtained and the ratio of the difference to the original weight was calculated.

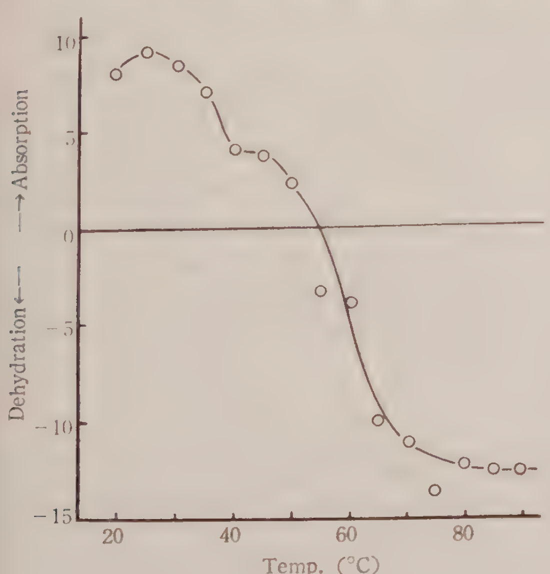


Fig. 17. Curves of temperature-ratios of absorption of water or dehydration

If the original weight (*A*) is less than the weight (*B*) of a block after the soaking, the ratio shows minus; this is the ratio of absorption of water. But if the original weight (*A*) is larger than the weight (*B*) of block after the soaking, the ratio shows plus ; this is ratio of dehydration.

At each temperature, each ratio was plotted on a graph and curves were drawn of temperature-ratios of absorption of water or dehydration.

2) Experimental results

Results obtained are graphed in Fig. 17.

As seen in Fig. 17, with the boundary at 55°C, the meat absorbs water below 55°C, and it dehydrates above 55°C. In the part of the curve showing absorption of water, there were two stages. The first step of the absorption ended at 40°C; the second step began at 50°C and ended at 65°C. In the part of curve showing dehydration, above 70°C the ratios of dehydration showed almost constant. At this point, all the proteins in the meat seemed to be completely coagulated, and the ratio of dehydration attained to the maximum. On the basis of those observations, there seem to be two kinds of proteins in the "Suketodara" meat. One of them corresponds to myosin and the other corresponds to myogen.

(2) **Heat coagulation**

When fish meat is heated in water, the proteins in the meat are coagulated at a certain temperature according to the kind.

1) Experimental method

Fresh "Suketodara" was filleted. The skin was removed from the meat, and the meat was homogenized to make a sample. To 100 g of the homogenized meat 300 cc of dist. water was added. The mixture was left for one hour with occasional agitation. After having been left, the mixture was filtered. Ten cc portions of the filtrate (water-soluble protein) were poured separately into test tubes. Those test tubes were heated separately at each definite pre-determined temperature from 35°C to 85°C for 10 minutes. After the heating, the test tubes were cooled rapidly, and the solution was filtered. The total amount of nitrogen in 5 cc of the filtrate was estimated by Kjeldahl's method. The difference in the amount between that in a definite volume (5 cc) of the filtrate (non-coagulable protein) and in that of the original water-soluble protein solution was considered to be the coagulable protein. Similarly the amounts of coagulable protein in the meat heated in 0.5 Mol NaCl solution at various temperatures was estimated.

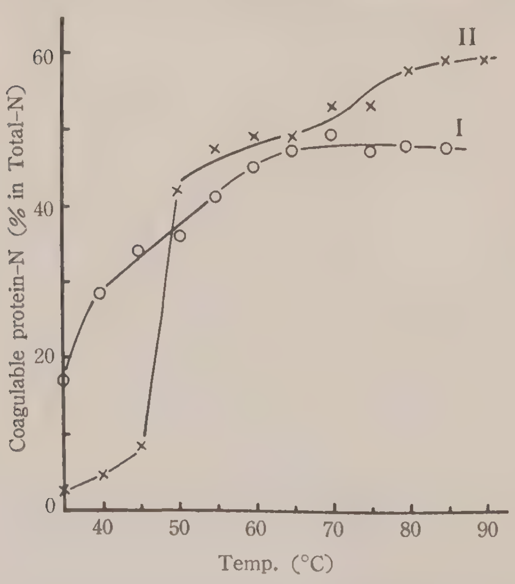


Fig. 18. Curves showing the changes of the amounts of heat coagulable protein of "Suketodara" (*Theragra chalcogramma*) meat

I ···Water soluble protein

II ··NaCl solution soluble protein

2) Experimental results

Results obtained are shown in Fig. 18.

As seen in Fig. 18, the heat coagulation of water-soluble protein was completed at 60° ~ 65°C. At the coagulating point, the amounts of the protein were constant

showing as 45 ~ 47 % of the total water-soluble protein.

The curve showing the water-soluble protein ascended gradually from about at 35°C, and became constant at 60° ~ 65°C. The main component of the coagulable protein in this case is considered to be myogen.

On the other hand, the heat coagulation of NaCl solution-soluble protein is continued gradually from 35° to 45°C, but it is continued rapidly from 45°C to 55°C and it becomes slow from 60°C. The coagulation is again caused to occur pretty rapidly from 70°C to 75°C. That is to say, there are observed in this curve two steps indicating the presence of two kinds of proteins. One of the proteins which is coagulated at the lower temperature is actually myosin, while the other at higher temperature is considered to be myogen. The presence of two kinds of proteins was also observed in the absorption curve obtained as described in the previous article.

7. Hydration of the "Suketodara" meat

In order to find out about the hydration of "Suketodara" meat, the water-content (g) — relative vapour pressure (p/p_0) curve in the fresh raw meat was determined, and compared with that of other kinds of fish meat.

Similarly the curves in frozen "Suketodara" meat prepared by rapid or slow freezing methods which was stored during various periods at low temperature were determined, and the relation between hydration and freezing or storing was ascertained.

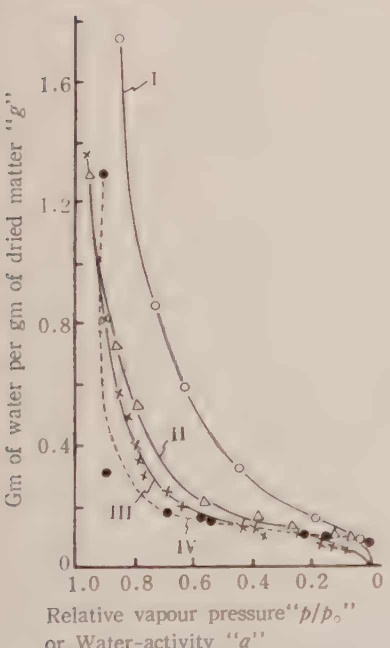


Fig. 19. Comparison of "$g—p/p_0$" curve of the "Suketodara" meat with that of other kinds of fish meat

I ···Suketodara
II ···Atka mackerel
III ···Squid
IV ···Sea-cucumber

(1) Fresh raw "Suketodara" meat

1) Experimental method

The relation between water-content (gram of water per 1 g of the dried matter), "g" and relative vapour pressure (p/p_0) in fresh "Suketodara" meat (water-content 81.18 %, volatile basic nitrogen 5.34 mg%) was determined at 20°C by vapour tension method[10].

2) Experimental result

The result obtained from the fresh raw "Suketodara" meat is shown as curve I in Fig. 19. In the same Fig., curve II is for Atka mackerel (20°C)[7], curve III is for squid (20°C)[10]; curve IV is for sea-cucumber meat (18°C)[2].

As seen in Fig. 19, when water in each sample shows the same relative vapour pressure (that is water-activity "*a*"), the hydrating affinity decreases in the order "Suketodara", Atka mackerel, squid and sea-cucumber. That is to say, the hydrating affinity is the strongest in the raw meat of "Suketodara".

(2) **Frozen "Suketodara" meat**

1) Experimental method

Several filleted meat samples of fresh raw "Suketodara" were divided into two groups. One group was rapidly frozen at -29°C in a contact freezer, and the other was slowly frozen at -17°C in an air-freezer.

After freezing of each sample, they were enveloped in polyethylene paper and placed in cold storage at -17°C for definite periods (22, 50 and 90 days).

After the storing, the amounts of water, nitrogen and drip were estimated. At the same time, the relation between water-content and relative vapour pressure was estimated by the same method as that described above.

2) Experimetal result

The results of chemical analysis are shown in Table 17. The curves showing water-content—relative vapour pressure ("g-p/p_0") are shown in Figs. 20~22.

Table 17. Chemical results of analysis of frozen and stored "Suketodara" meat

Items \ Freezing method \ Storage	0	22 days		50 days		90 days	
	Raw meat	Quick Freezing	Slow Freezing	Q.F.	S.F.	Q.F.	S.F.
Total-N (%)	2.35	2.67	2.63	2.56	2.54	2.58	2.48
Water content (%)	81.18	79.87	80.23	80.44	81.59	79.98	83.75
Protein-N (%)	2.26	2.36	2.33	2.36	2.23	2.29	2.15
V.B.-N (mg%)	5.34	8.41	8.67	10.01	10.41	15.35	16.02
pH of the meat	6.2	6.0	6.2	6.2	6.3	6.4	6.4
H_2O-soluble-N (% in Total-N)	37.2	34.7	28.4	28.9	26.2	29.8	26.2
0.5M.NaCl-soluble-N (% in Total-N)	46.0	45.2	36.9	26.37	30.98	33.64	28.00
Free drip (%)	0	10.3	6.7	9.4	8.9	10.8	12.8
Expressible drip (%)	—	6.4	15.5	10.8	21.9	20.8	22.5

As seen in Table 17, during the cold strage, the solubilities of "Suketcdara" meat by water or 0.5 Mol NaCl solution decreased, while the amount of drip increased.

The decreasing of the solubilities of the meat by various solutions and increasing of drip are larger in the slow frozen meat than in the rapid frozen meat. This suggests that the denaturation of meat protein and destruction of the tissue are larger in the meat frozen by slow freezing.

As seen in Figs. 19 and 20, the hydrating affinity of water in the frozen meat

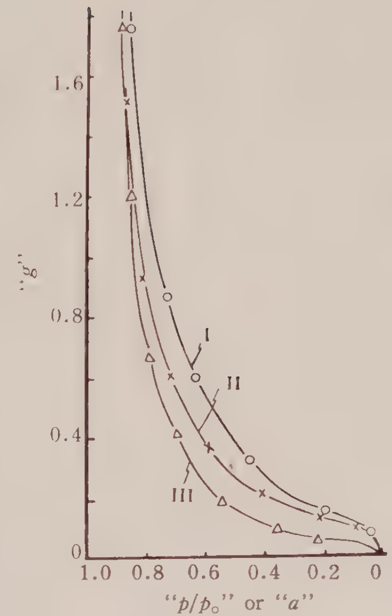


Fig. 20. Curves showing "g—$p/p_\circ$" of slow frozen "Suketodara" meat

I ...... Fresh raw meat
II ...... Frozen meat (22 days storing)
III ...... Frozen meat (90 days storing)

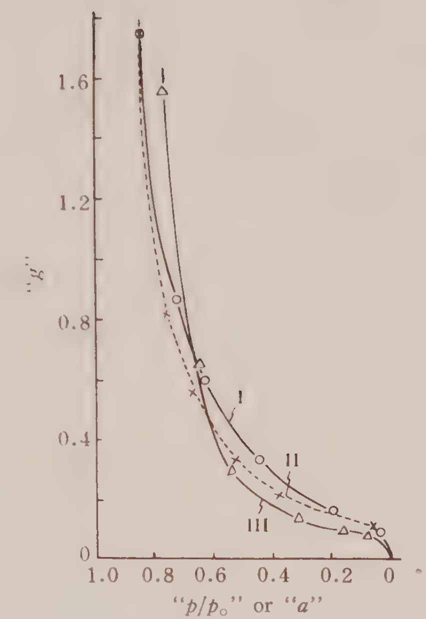


Fig. 21. Curves showing "g—$p/p_\circ$" of rapid-frozen "Suketodara" meat

I ...... Fresh raw meat
II ...... Frozen meat (22 days storing)
III ...... Frozen meat (90 days storing)

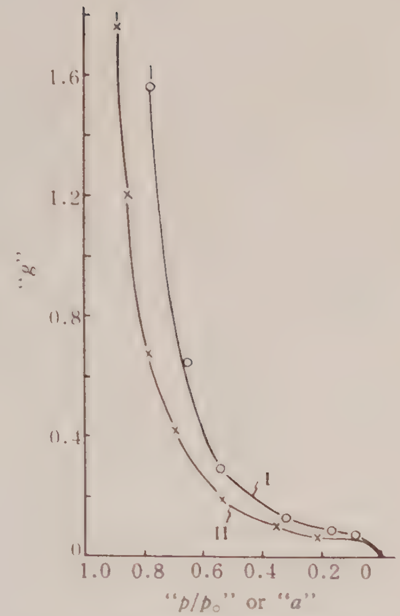


Fig. 22. Comparison of curves of "g—$p/p_\circ$" of "Suketodara" samples prepared by rapid and slow freezings

I ...... Rapid frozen meat (55 days storing)
II ...... Slow frozen meat (55 days storing)

became smaller than that in the fresh raw meat when the comparison was made at the same water activity "*a*" (that is p/p_0). In meat frozen by the same freezing method, the hydrating affinity of water in the frozen meat which was stored for a longer period became smaller than in that stored for a shorter period. As seen in Fig. 21, the hydrating affinity of water in slow-frozen meat was smaller than in that frozen by rapid freezing at the same water activity "*a*".

The result obtained as above will suggest that the hydrating affinity of water in fish meat decreases with the denaturation of the meat protein during cold storage.

General Discussion

The Suketodara-fish which are abundantly caught in Hokkaido, Japan, are employed to process for dried goods (*e.g.* dried "Suketodara" after freezing, "Mintai"), or for salted and dried goods (*e.g* filleted dried "Suketodara", "Sukimidara").

However, recently with the prosperity of the processing of fish sausage or fish ham the "Suketodara" meat has also attracted attension as a additive raw material for them.

Like Japanese fish paste, such as "Kamaboko" or "Chikuwa", fish sausage and fish ham are manufactured by homogenizing two or more kinds of fish meat with salt, and by heating to coagulate. The first physical necessity as the raw material for fish sausage and fish ham or Japanese fish pastes is strong elasticity of the meat pastes when the raw materials are processed.

Gengerally speaking, "Suketodara" meat as a raw material shows comparatively weak elasticity of the pastes[11]. Before estimating the value as the raw material for fish pastes, the authors undertook a fundamental investigation of the properties of the meat of "Suketodara".

In comparison with other fishes (*e.g.* Atka mackerel[7] etc.), "Suketodara" meat has scarce fat and protein (See above Chapter Ⅰ, Articles 1 and 2[1]).

On the other hand, the kinds of proteins of the "Suketodara" meat and the nitrogen distribution in the meat extractive and the composition of amino acids in the protein are almost the same as corresponding characters of other fishes (See Chapter Ⅰ, Article 3).

Therefore, the more plain taste of the "Suketodara" meat than that of other fish meat is not attributable to the composition of amino acids. Shimidu[11] has said that the taste rapidly becomes worse with falling of freshness. This will be explained somewhat by the more rapid velocity of fall in freshness of the "Suketodara" meat than that of other fishes (See Chapter Ⅱ[1]). On the other hand, the more plain taste will also be explained by the scarce fat and low solubility of nitrogeneous components by 0.6 Mol NaCl solution (*e.g.* about 40% for "Suketodara" meat, and about 50% for Atka mackerel) (Chapter Ⅲ, Articles 1, 3, 4), or by the facts of the histological properties in which the muscle tissue is comparatively fine and the amount of body fluid components between connective

tissues seems to be small (Chapter II, Article 4[1]).

The fact that successively extracted solutions of "Suketodara" meat with Weber's solution or 0.6 Mol NaCl solution did not show the phenomenon of flow birefringence is peculiar, as compared with other fish meat [illegible]. This fact may perhaps be due to small quantity or different quality of myosin which is the cause of the phenomenon of flow birefringence considering from the fact that the presence of myosin was confirmed by the results from the experiments on the dehydration, hydration or heat coagulation (Chapter III, Article 6). As the elasticity of the fish pastes is said to be influenced remarkably by the quantitative difference of myosin (actomyosin) in fish meat protein[1], the weak elasticity of fish pastes which are processed from "Suketodara" meat will be due to the peculiarity of the meat protein. This point must be further investigated in the future.

As to the phenomenon of swelling of the "Suketodara" meat in various concentrations of various salt solutions, the maximum swelling was observed at 0.4～0.6 Mol in solutions of monovalent salts, and at 0.2 Mol in solutions of bivalent salts (Chapter III, Article 5). In solutions of bivalent salts, when concentration of the solutions is above 1 Mol, the swelling decreases in the following order, $NH_4^+ > K^+ > Mg^{++} > Ca^{++}$ as to cations, and $I^- > NO_2^- > SO_4^{--} > Cl^- > CNS^-$ as to anions.

The results for tests on the swelling of "Suketodara" meat almost agreed with Fujii's results for Atka mackerel meat[7].

Although as to the relation between pH and the swelling of fish meat, the minimum swelling is said to be shown in the range of the isoelectric point of the meat protein, the minimum swelling of the "Suketodara" meat was not clearly shown at pH 5.0～5.4 which is in the range of the isoelectric point (Chapter III, Article 2). This perhaps is due to the reason that on the acidic side of the isoelectric point, the acid coagulation of the meat is too strong to show the swelling and the swelling will be restricted.

Therefore, this fact suggests that care must be taken in acidic treatment during the processing of fish paste.

When the "Suketodara" meat is heated in water at various temperatures, it shows absorption of water below 55°C, and dehydration above 55°C (Chapter III, Article 6). On curves showing absorption or dehydration of water there are two steps; this suggests that there are two kinds of proteins in the "Suketodara" meat; one is a group of proteins coagulated at low temperature (about at 40°C), and the other is that coagulated at comparative high temperature (about 65°C); the former agrees with the coagulating temperature of myosin while the latter agrees with the coagulating temperature of myogen.

The dehydrating ratio of "Suketodara" meat at high temperature (above 80°C) is smaller than that of other fish meat. (*e.g.* "Suketodara" meat was about 12%, Atka mackerel meat was about 20%). The point at which the absorption changes to dehydration in the Atka mackerel meat was about 37°C, on the other hand, that in "Suketodara"

meat was about 55°C. This observation will show that the "Suketodara" meat is difficult to dehydrate at low temperature.

Those facts will show that the amount of myosin in meat protein of "Suketodara" is small, and the absorption or dehydration is remarkably influenced by the heat-coagulability of myogen in the meat. Considering that squid or sea-cucumber meat which have coarse muscle tissue and a large amount of connective tissue showed great dehydration (each about 35 and 60%), the "Suketodara" meat of which the muscle tissue is compact, seems to have a small amount of dehydration caused by heating.

Further peculiar properties are observed in hydration of the "Suketodara" meat besides the properties of the proteins or histological structure (Chapter Ⅲ, Article 7). When the curve showing water-content (g) — relative vapour pressure (p/p_0) relation in "Suketodara" meat was compared with that of other fish, the amount of the hydrating affinity decreased in the order of "Suketodara", Atka mackerel, squid and sea-cucumber at the same relative vapour pressure (p/p_0) (*i.e.* at the same water activity, "a"). This observation was contrary to the supposition that the "Suketodara" meat having scarce fat and large amount of water-content would at first have a large amount of free water. The authors have considered that the difference or order of the bound water in meats of various kinds of fish may be due to qualititative or quantitative differences in tissue proteins. But this point must be further investigated in the future.

Summary

Before investigating the utilization of the meat of "Suketodara" which are caught abundantly in Hokkaido, Japan, the fundamental properties of the meat have been studied. Results obtained are summarized as follows:

(1) The "Suketodara" meat belongs to that type of fish meat having a large amount of water-content, but scarce fat and protein, although the chemical components of the meat vary somewhat by fishing season, six and size.

(2) The nitrogen distribution and the composition of amino acids in meat of the "Suketodara" are almost the same in comparison with other kinds of fish meat.

(3) The "Suketodara" meat falls more rapidly in freshness than other fish meat. Especially the incipient falling velocity of freshness of the "Suketodara" meat is more rapid than that of other fish meat.

(4) The "Suketodara" meat has muscle tissue with comparatively fine muscle fibre showing fine histological construction.

(5) With the falling of the freshness the amount of bound water in fish meat decreases.

(6) The amount of dissolving out of nitrogenous components in water or saline solution is smaller from "Suketodara" meat than that from the other fish meat.

(7) The isoelectric points of water soluble protein and salt solution-soluble protein are almost similar (both range in pH $5.0 \sim 5.4$).

(8) In the solution extracted by salt solution (Weber's solution or 0.6 Mol NaCl solution), the phenomenon of flow birefringence was not observed. This is perhaps due to the comparatively smaller amount of myosin.

(9) The swelling of the "Suketodara" meat in various kinds of salt solutions little few difference from that of other kinds of fish meat.

(10) As to the relation between pH and the swelling of "Suketodara" meat, the swelling was restricted in the acidic side of the isoelectric point, differing from other kinds of fish meat.

(11) Dehydration of "Suketodara" meat by heating in water is difficult. From curves of absorption of water, dehydration or heat-coagulation, there seem to be present two kinds of proteins: myosin and myogen.

(12) Water in the "Suketodara" meat has good affinity to the meat proteins. The amount of water hydrated at the same water-activity "*a*", is larger than that of other kinds of fish meat.

(13) The hydration decreases with the denaturation of meat protein during freezing. The velocity of decrease is larger in slow frozen meat than in rapid frozen.

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ミズタコ肝臓の脂質

第3報 不鹼化物成分について

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Lipids from the Liver of *Octopus dofleini*

Ⅲ. On the Unsaponifiable Matter.

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Abstract

The unsaponifiable matter is composed 50.7 % of sterol, which consists mainly of cholesterol; it contains besides sterol such alcohols as tetradecanol, hexadecanol and octadecanol of the saturated series and tetradecenol, hexadecenol and octadecenol of the oleyl series, also such monoglycerylether as monooctadecylglycerylether and monooctadecenylglycerylether; the presence of a small amount of hydrocarbons is also presumed.

緒　　言

軟体動物頭足綱の肝臓脂質の不鹼化物成分に関する研究は脂肪酸のそれに比してあまり多くはなく，スルメイカ（*Ommastrephes sloani pacificus*）について辻本[1]，湊[2]がコレステロール，ヘキサデカノール，オクタデセノール，キミルアルコール，バチルアルコール，セラキルアルコール等が存在することを認め，ヤリイカ（*Loligo bleekeri*）についても辻本[3]がスルメイカとその構成成分が同一であることをみている。

同綱のマダコ（*Octopus vulgaris*）については辻本ら[4]がスクアレンは存在しないことを認め，湊[2]がバチルアルコールとコレステロールの他に一つのステロール性物質が存在することをみている。また松本ら[5]はミズダコ（*Octopus dofleini*）にはコレステロール（59.1 %）とオクタデカールが存在することを報告している。

著者はミズダコ肝臓脂質について系統的検索を試み，前報[6,7]ではアセトン可溶性脂質の脂肪酸組成について報告したが，本報ではその不鹼化物成分について検索を行い，コレステロールの他にテトラデカノール，テトラデセノール，ヘキサデカノール，ヘキサデセノール，オクタデカノール，オクタデセノールなどの脂肪族一価アルコールとバチル，セラキルアルコールなどのモノグリセリールエーテルを認め，炭化水素の存在を推定したので報告する。

実験及び結果

不鹼化物成分の抽出，濃縮の如き操作は全て減圧下 CO_2 気流中で行い，沃素価は Wijs 法，ステロール含量はジギトニン法によって測定した。

試料としては前報[6]において用いたミズダコ肝臓アセトン可溶性脂質（Table 1）1 kg を鹼化して得た粗不鹼化物を用いた。

(1) 不鹼化物成分の分割

アセトン可溶性脂質から混合脂肪酸を調製する際に得られた不鹼化物を 2 N エタノール性 KOH で再鹼化したのち，エーテル抽出，水洗，乾燥後，エーテルを溜去して鱗片状結晶を混じえた暗赤色軟質の固体の不鹼

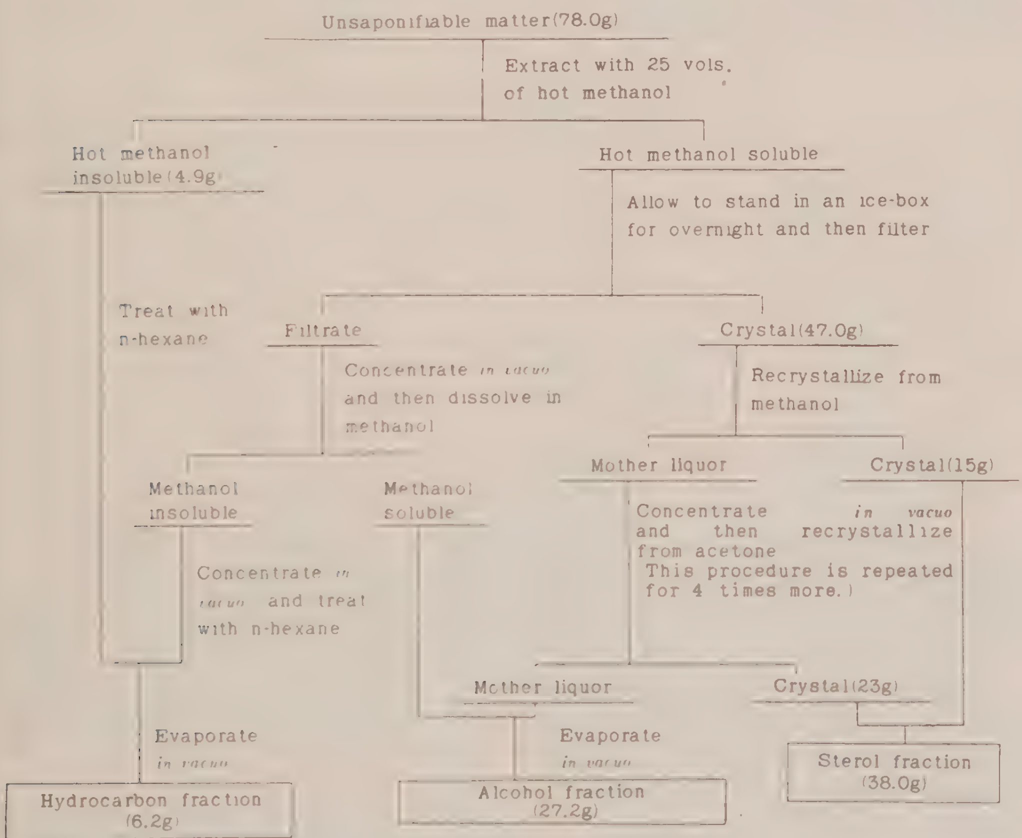
Table 1. Properties of acetone-soluble lipid from the liver of *Octopus dofleini*

Appearance	Dark brown liquid	Acid no.	77.3
Yield, % from fresh tissue	14.6	Saponification no.	174.9
Specific gravity, d_4^{20}	0.9323	Iodine no.	201.0
Refractive index, n_D^{20}	1.4833	Unsaponifiable matter, %	8.57

化物78.0gを得た。このものはアセチル価142.7，沃素価130.7，融点95—98°，ステロール含量は不鹸化物中50.70％であつた。

この不鹸化物を Fig. 1 に示す如く溶剤を用いて分劃し，各ステロール劃分，アルコール劃分及び非アルコール（炭化水素）劃分に分劃した。

Fig. 1. Fractionation of the unsaponifiable matter



(2) ステロール

Fig. 1に従つて分劃し，白色針状結晶のステロール性物質（融点145—146°，Lieberman-Burchard 反応陽性）38.0g を得た。これを無水エタノールを溶媒として紫外部（240—300mμ）における吸収を測定した結果，共役ステロール（$\Delta^{5,7}$-ステロール）の吸収極大を示さず共役ステロールは存在しないことが認めら

れた。

この粗ステロールはアセトンから再結晶をくりかえして融点146—147°，$[\alpha]_D^{15}$-39.0 ($CHCl_3$) のものを得た。これは大部分がコレステロール（文献値：融点 148—149°，$[\alpha]_D^{15}$-39.5 ($CHCl_3$)）と考えられる。なおこのものを確認するために一部を2倍量の無水酢酸と共に加熱してアセチル化し，95%エタノール及びアセトンで再結晶を行い，融点113—114°，$[\alpha]_D^{15}$-46.1 ($CHCl_3$) のものを得た。このものの母液部濃縮物の融点は 113—114° でありコレステロール酢酸エステル（文献値：融点 113—114°，$[\alpha]_D^{15}$-47.5 ($CHCl_3$)）と一致した。また一部をピリジン溶液中でベンゾイルクロライドでベンゾイル化したものを95%エタノール，アセトンから再結晶をくりかえして融点 146—147°，$[\alpha]_D^{15}$-14.3 ($CHCl_3$) のものと母液部濃縮物の融点 145—146° のものを得た。このものはコレステロール安息香酸エステル（文献値：融点 147°，$[\alpha]_D^{15}$-14.0 ($CHCl_3$)）と一致した。

(3) アルコール及びモノグリセリールエーテル

Fig. 1 の如くして得たアルコール劃分 27.0g は赤橙色軟質固体を混じえた液体で，アセチル価 233.1，沃素価 79.6，Lieberman-Burchard 反応陽性であつた。このものをアセチル化してその 20g（アセチル化物の沃素価 62.0）を精密に分溜し Table 2 に示す結果を得た。

Table 2. Fractional distillation of the acetate of alcohol

Fraction no.	B. P. °C /1.5mmHg	Yield g	Yield %	Sapon. no.	Iodine no.	Refractive index, n_D^{20}	Lieberman-Burchard Reaction
1	-130	0.9	4.5	210.6	52.7	1.4587	–
2	130-180	0.7	3.5	200.7	55.1	1.4592	–
3	180-210	2.4	12.0	180.6	55.4	1.4605	–
4	210-220	5.2	26.0	216.7	56.2	1.4670	±
5	220-225	4.7	23.5	259.1	56.6	1.4672	+
6	225-237	2.7	13.5	201.2	96.3	1.4918	++
7	Residue	3.4	17.0	123.7	105.4	—	++

溜分1は鹸化価よりみて C_{14}とC_{16}アルコールアセチル化物よりなるものと考えられ，この溜分をラネーニッケルを触媒とし無水エタノール溶液中，水素添加した生成物を鹸化して得た淡黄色結晶は融点 40—41°，アセチル価 206.9で，80%メタノールより分別結晶を行い， 融点46—48°の結晶と融点30—34°の母液部濃縮物を得た。これらのものは少量のため精製不充分であつたが恐らくテトラデカノール（融点 38°，理論アセチル価 219.0）とヘキサデカノール（融点 49°，理論アセチル価 197.3）にそれぞれ該当するものと考えられる。従つて原溜分は沃素価の点から考えてテトラデセノール（理論アセチル価 220.7，理論沃素価 119.6，アセチル化物の理論沃素価 99.8）とヘキサデセノール（理論アセチル価 198.8，理論沃素価 105.7，アセチル化物の理論沃素価 89.9）も含まれていると考えられる。

溜分3は C_{18}アルコールアセチル化物よりなるものと考えられ，一部をアセトン溶液中で $KMnO_4$ 酸化して得た飽和アルコールアセチル化物を鹸化後エタノールより再結して得た結晶は，融点 57—58°，アセチル価 179.0 であつた。また一部を水添したものを脱色炭で処理して飽和アルコールアセチル化物を得た。鹸化後エタノールより再結を行つて得た結晶は融点 57°，アセチル価 179.7 で，それぞれオクタデカノール（融点58°，理論アセチル価 179.6）に一致した。

従つて原溜分はオクタデカノールの他にオクタデセノール（理論アセチル価 180.8，理論沃素価 94.6，アセチル化物の理論沃素価 81.8） よりなるものと思われる。

溜分 5 はバチル，セラキルアルコールとコレステロールの各アセチル化物よりなるものと考えられ，一部を $KMnO_4$ 酸化処理して得たものを鹸化し，エタノールより再結を行つた結晶は融点 70—71°，アセチル価 259.8 であつた。また一部を水添，脱色炭処理したアセチル化物を鹸化して得た結晶をエタノールより再結をくりかえして融点 68—70°，アセチル価 258.0 の結晶を得た。これらのものはそれぞれバチルアルコール（融点 70—71°，理論アセチル価 261.9）であることを認めた。原溜分はバチルアルコールとセラキルアルコール（理論アセチル価 263.2，理論沃素価 74.2，アセチル化物の理論沃素価 59.5）とが混在しているものと考えられる。

溜分 7 をアセトンより再結をくりかえして融点 137—139°，アセチル価 141.4 の結晶を得た。これはコレステロール（融点 148°，理論アセチル価 131.0）に該当しているので，原溜分はコレステロールの他にアルコール類が混在しているものと思われる。

(4) 非アルコール性物質（炭化水素）

Fig. 1 の如く分劃して得た非アルコール性物質 6.2g を再鹸化し，石油エーテルで抽出した物質は褐色粘稠性の液体（5.7g）でアセチル価 6.5，沃素価 80.2，n_D^{20} 1.5030 であつた。この結果アセチル価が非常に低い値を示していることより炭化水素が主成分であることが推定され，更に沃素価が 80.2 であることより不飽和炭化水素も含まれていることが推測された。

考察及び総結

ミズダコ肝臓アセトン可溶性脂質より不鹸化物を分離し，溶剤分劃法によつて各劃分に分劃し，ステロールは誘導体を生成して確認し，アルコール類は分溜法によつて検索を行つた。

ステロールは不鹸化物中 50.7% を占める主成分であり松本ら[5]の結果 59.1% とほぼ一致した。また紫外部吸収の結果からしても $\Delta^{5,7}$-ステロールの如き共役ステロールも認められず，コレステロールのみであつた。その他[illegible]等の報告にあるステロール性物質（アセチル化物の融点118°）の存在は本試料中には全く認められなかつた。

ステロール以外の脂肪族アルコールとしては，オクタデカノール，オクタデセノールの含量が比較的著量であり，次いでヘキサデカノール，ヘキサデセノールが存在し，その他テトラデカノール，テトラデセノールの存在も認められたが，その含量は極めて少いようであつた。

またバチルアルコール（モノオクタデシルグリセリルエーテル）とセラキルアルコール（モノオクタデセニールグリセリルエーテル）のグリセリルエーテルも著量に存在したが，キミルアルコール（モノセチルグリセリルエーテル）の存在は認められなかつた。

この傾向はスルメイカ，ヤリイカ，マダコ等の肝臓不鹸化物成分[1,2,3,4]と比較的類似している。

そのほかにメタノール不溶の非アルコール性物質も存在し，これはアセチル価 6.5 という非常に低い値を示していることより恐らく炭化水素であろうと思われ，更に沃素価 80.2，n_D^{20} 1.5030 という比較的高い値を示していることより不飽和結合を有するものも含まれていることが推定された。これらは何れの機会に再検討したいと考えている。

本研究遂行に当り[illegible]教授，[illegible]助教授の御指導と御鞭撻に対し厚く感謝の意を表します。

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[illegible]肝油中の炭化水素

辻　野　勇

（[illegible]）

Hydrocarbons in Pollack Live Oil

Isami TSUJINO

Abstract

A study has been made of the hydrocarbons in pollack liver oil. Squalene and heptacosane are the principal hydrocarbons.

Unsaponifiable matters (196g) obtained from low-boiling fraction (200-250°C/10^{-3}mm) of molecular distillation, were dissolved in acetone to remove the most part of cholesterol (97.8g). The acetone soluble matters were treated with methyl alcohol and methyl alcohol insoluble fractions were separated by alumina column chromatography. From the non-adsorbed fractions (11.5g), thin plate crystals (0.20g), m. p. 57-58°C, were obtained, possibly heptacosane. Mother liquids were fractionated under reduced pressure followed by alumina column chromatography, as shown in Tables 2 and 3. Squalene (1.48g) was isolated in a pure state and identified as of squalene hexahydrochloride.

肝油中に存在する炭化水素については辻本[1]がスクアレンを発見して以来多くの研究が行われている。タラ科肝油中の炭化水素については中宮・川上[2]が水素添加により，ノナコサンを分離し，原物質は$C_{29}H_{48}$のような不飽和化合物であるとし，また中宮[3]は不飽和炭化水素ガドセン($C_{18}H_{32}$)が存在すると報告している。[illegible]Drummond[illegible]塩化水素加成物として不飽和炭化水素を分離しスクアレンであると報告している。一方川上[6]は不ケン化物のメタノール不溶部よりナフタリン系と思われる$C_{13}H_{14}$，$C_{14}H_{16}$に相当する液体を得ている。また上野・小森[7]はn_D^{20} 1.4783の炭化水素を分離しているが[illegible]については研究していない。

[illegible]を行っている。ウバ鮫肝油のこの留分については先に報告[8]したが，スケトウタラ肝油の場合も酸価及び不ケン化物[illegible] 1 [illegible]研究試料として好適である。著者は北海道肝油工業協同組合の好意によりこの留分を入手し，メタノール分別及びアルミナカラムクロマトグラフィーにより炭化水素を単離検索したので報告する。

実験及び考察

[illegible]20kg 1[illegible] 200－250°C/10^{-3}mm の留分[illegible]暗黒色の軟膏状固体。Table 1に示す特数を有する。

[illegible]1.56[illegible]100g[illegible]合計196gの不ケン化物を得た。常温で固体，赤褐色。

Table 1. Properties of pollack liver oil

Acid value	114.5	Iodine value	147.1
Saponification value	156.6	Unsaponifiable matter	19.4%

コレステリンの分離：不ケン化物を2分しそれぞれアセトン500ccより再結し，少量の冷アセトンで洗いmp141～143°Cの結晶合計69.9gを得た。アセトンより更に再結するとmp143～145°Cを示し，また常法通りアセテートを作るとmp111～112°Cを示すのでこの結晶はコレステリンと考えられる。上記のロ液及び洗液は合一しアセトンを留去すると更に20.1gの粗コレステリンが得られる。

コレステリンを除去した黒色粘稠の残留物は再ケン化し，不ケン化物を集め放置すると粘稠液を含んだ結晶7.8gを分離する。アセトンより再結するとmp142～143°Cを示す。

メタノール処理：コレステリンを分離した粘稠なロ液85gをメタノール100ccと加熱し，冷後メタノール不溶部を集め，更に同様にメタノール処理をくりかえし合計600ccのメタノールで処理し可溶部50.5g，不溶部33.7gを得た。

アルミナクロマトグラフィー：メタノール不溶部28gを石油エーテル（60°C以下）200ccに溶解し，活性アルミナ（和光製）のカラム（30×570mm）を通し，次に石油エーテル700ccで展開すると色素及びアルコールは4個のゾーンとなつて吸着される。カラムを通過した部分より溶剤を追出し淡黄色の液体11.5gを得る。冷却すると少量の結晶が析出する。

固体炭化水素（ヘプタコサン）の分離：上記のカラム通過部は冷却しながらロ過を行いmp46～50°Cの白色薄板状結晶0.20gを得た。アセトンより5回再結し，P_2O_5上で乾燥。分析の結果は炭化水素である事を示し，mpの文献値よりヘプタコサン（$C_{27}H_{56}$）であると考えられる。

試　料	C% 85.02	H% 14.64	mp 57～58°C
$C_{27}H_{56}$として	85.26	14.73	59°C

スクアレンの分離：ヘプタコサンを分離したロ液9.5gはTable 2に示すように分留した。

Table 2.　Fractional distillation of liquid hydrocarbon

Fraction No.	Bioling point °C/1.5 mm	Yield g	n_D^{15}	Iodine value
1	117-175	1.20	1.4562	59.4
2	175-209	2.35	1.4862	201.7
3	210-220	4.80	1.4942	317.2
4	220	0.30	1.4960	292.7

留分1は冷却しても液状を保つ。メタノールと数回加熱し，不溶部を集め，石油エーテル中で濃硫酸と無色になるまで振る。n_D^{15} 1.4510，n_D^{20} 1.4496　更に発煙硫酸及び濃硫酸で振ると n_D^{15} 1.4460，n_D^{20} 1.4448 となる。恐らくプリスタン（n_D^{15} 1.4410，n_D^{20} 1.4390）のような飽和液状の炭化水素が存在すると思われる。

留分2は常温で結晶をまじえた液体で，ヨウ素価より高度不飽和の炭化水素の混在すると考えられる。

留分3は留分2と同様結晶が析出しているが，ロ過によつて完全に取り除くことは出来ない。よつて4.3gを石油エーテルに溶解して不溶物を除き，アルミナカラム（25×200mm）を通し，石油エーテルで溶出し20ccづつ分割した。

結晶性固体炭化水素は液体炭化水素よりも早くカラムを通過する。石油エーテルを追出すと1～3は溶出物ないが，4は白色の結晶をまじえた無色の液体，5以下は無色透明の液体が得られるがn_Dが近似していて同一物と考えられるので合一した。この結果をTable 3に示す。

留分4は結晶が析出し，ヨウ素価より前記ヘプタコサンと高度不飽和炭化水素の混合物と思われる。留分5～21は分子量及びヨウ素価は$C_{30}H_{50}$によく一致するのでスクアレンと思われる。

Table 3. Chromatographic separation of highly unsaturated fraction, using alumina

Fraction No.	Yield g	n_D^{14}	Iodine value	Molecular weight*
4	1.42	1.4820	251.1	—
5-21	1.48	1.4967	348.7	395
$C_{30}H_{50}$			371.1	410

* Benzol, cryoscopic method

更にこれを確認するため留分 5～21 0.417 g をエーテル中で冷却しながら乾燥塩化水素ガスを通し，白色結晶 0.195 g を得た。アセトンより 4 回再結したものは 108°C より融けはじめ 118°C で透明となる。

試 料	C% 57.46	H% 8.87	Cl% 33.88
$C_{30}H_{50}\cdot6HCl$として	57.22	8.97	33.81

元素分析の結果もまたスクアレンであることを支持しており，ガドセン（$C_{18}H_{32}$，分子量 248，ヨウ素価 307；$C_{18}H_{32}\cdot3HCl$，C 60.39%，H 9.86%，Cl 29.74%）は検出出来なかつた。

総　　括

スケトウタラ肝油中に存在する炭化水素について検索した。不ケン化物よりアセトン再結を行つてコレステリンを除き，メタノール処理，アルミナクロマトグラフィー及び減圧分留により 2 種の炭化水素を単離した。固体炭化水素 mp 57～58°C はヘプタコサンと思われ，液体炭化水素は分子量，ヨウ素価さらにハイドロクロライドの分析値よりスクアレンと決定した。このほかに微量の飽和液状の炭化水素も存在する。

本実験に当り御指導，御鞭撻を頂いた斎藤恒行教授，試料を提供して頂いた大沢清氏，実験に協力された望月英明氏，元素分析をお願いした大阪大学理学部有機微量分析室の各位に厚く感謝いたします。

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水産無脊椎動物筋肉中の酸可溶核酸成分 II

貝類筋肉中の酸可溶核酸成分に及ぼす貯蔵温度の影響 2

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II. Acid-soluble Nucleotides in Muscle of Marine Invertebrates

Effects of storing temperature upon the content of muscular nucleotides of some seashells (2)

Ken-ichi Arai

Abstract

The changes in the amounts of nucleotides, nucleosides, and purine bases during the storage of fresh muscles of some seashells (surf clam and scallop) have been followed by means of ion exchange chromatography: the results were reported previously[1].

The changes of the compounds found in the muscles of ark-shell and abalone during storage under different circumstances were described in this paper. The results of the study may be summarized as follows:

(1) Values of ATP, ADP, and AMP (μmole/g. muscle wet wt.) were 3.20-2.67, 0.97-1.27, and 0.35-1.21 for the fresh foot muscles of ark-shell (Anadara broughttoni Shrenk), 2.40-3.08, 0.38-0.50, and 0.15-0.25 for the fresh foot muscles of abalone (Haliotis discus hannai), respectively.

(2) During the cold storage of both sorts of fresh muscles at −5°C there was a decomposition of ATP accompanying the formation of ADP and AMP which was finally accumulated as time passed. A very small amount of adenine was found only in the muscle of ark-shell. Generally, the changes in the muscle of ark-shell were faster than in abalone.

(3) When samples of both sorts of fresh muscles were held at room temperature scarcely any changes could be observed for about 20 hours from the start. After that changes in the amounts of inosine-like compound were found only in the muscle of ark-shell.

水産無脊椎動物筋肉中の酸可溶核酸成分の本性を検討するため，著者は先に，ホタテ貝及びウバガイを試料として選び，筋肉中の酸可溶核酸成分量と貯蔵温度の関連について報告した[1]。

著者は更に，同じく軟体動物の貝類に属するアカガイ及びアワビの筋肉中の同成分と貯蔵温度の関連について検討した結果，明らかに先に報じたホタテ貝及びウバガイの場合と異なる特異な現象を認めたので，ここに報告する。

実 験 方 法

試料は函館近海産のアカガイ（*Anadara broughttoni*）及びアワビ（*Haliotis discus*）を用い，いずれも水揚後数時間以内で，必ず生存しているものを選んだ。筋肉の採取及びその後の処理については，前報に述

本研究は水産無脊椎動物筋肉中の有機窒素化合物に関する研究 第X報とする。なお研究費の一部を土屋靖彦教授担当（斎藤恒行教授分担）の文部省総合研究費（軟体動物のエキスに関する研究）によって支弁した。ここに感謝する。

べた[1]。酸可溶核酸成分の分析についても，先に述べた常法に依つた[2]。

実 験 結 果

新鮮なアワビ筋肉及び-5°Cで凍結貯蔵25日後の筋肉中の酸可溶核酸成分のイオン交換樹脂からの溶出ヒストグラフを第1図及び第2図に示した。第1図及び第2図において分画されたB区分を除くA，C，D，Eの4溶出区分の成分組成については先にホタテ貝及びウバガイについて報じたような理由で，それぞれ非核酸系化合物と推定される未知物質，Adenosine mono phosphate (AMP)，Adenosine diphosphate (ADP)，Adenosine triphosphate (ATP) である。ただし，アワビ筋肉について得られるB区分は，貯蔵時間の経過によつて全く増加しない上（第2図），はつきりした極大吸収を示さないから，Inosine, Hypoxanthine またはその他関連物質の存在を認めるわけにはゆかない。この現象は筋肉をより高温で放置する場合においても同じことであつた。アカガイ筋肉についても全く同じ傾向の溶出ヒストグラフが得られるが筋肉が，16～17°Cに放置されるときは，このB区分に 250/260=1.2 位であつて，250mμに極大吸収を示し，paper chromatography によつて Inosine と推定される物質が，また-5°Cで凍結貯蔵するときは，250/260=0.80～0.85 で，260mμに極大吸収を示し，paper chromatography によつて Adenine と確認される物質が，いずれも微量ではあるが溶出された。

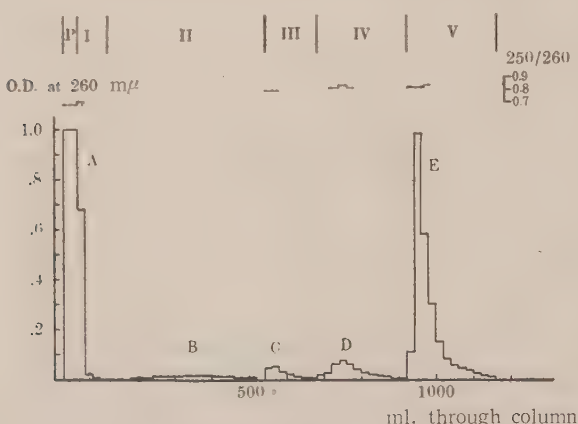


Fig. 1. Separation of acid-soluble nucleotides of the fresh foot muscle (abalone) by ion exchanger
Exchanger: Amberlite IRA-400, Flow speed: 1ml./min.
Solvent: P…Passed solution, I…0.01 M NH_4Cl+ 0.1 M NH_4OH, II…0.01 M NH_4Cl, III…0.005 M HCl, IV…0.02 M NaCl+0.01 M HCl, V…0.2 M NaCl+0.01 M HCl
A…Unknown compound, B…Unknown compound, C…AMP, D…ADP, E…ATP

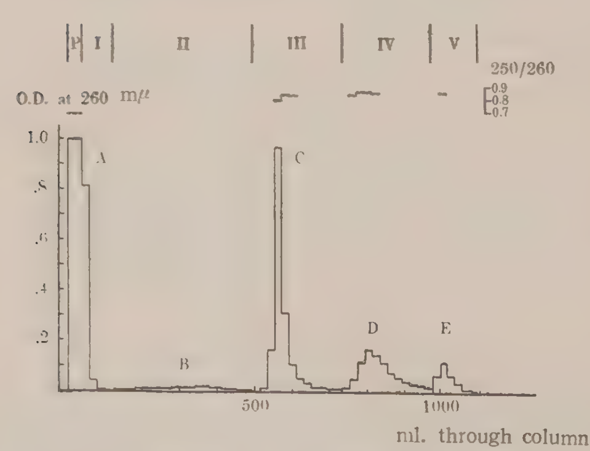


Fig. 2. Separation of acid-soluble nucleotides of the foot muscle (abalone) stored at -5°C. for 25 days, by ion exchanger
The experimental conditions are the same as those in Fig. 1.

A）アカガイ

アカガイは可食肉質部の中，いわゆる斧足とよばれる部分で，外観上均質となつている部分について分析を行つた。

新鮮筋肉を-5°C附近で緩慢凍結をする場合の変化を第3図に示した。第3図によると，新鮮な筋肉中の酸可溶核酸成分の主成分を成しているATPは，凍結貯蔵が12日間に及ぶと徐々に分解し，ADP次いでAMPが分解生成物として生成，蓄積することがわかつた。このAMPの蓄積は一方的であつて，微量の Adenine が見出される他は，Adenosine, Inosine, Hypoxanthine

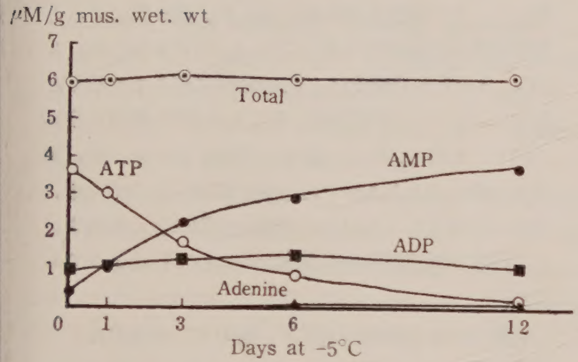


Fig. 3. Changes in adenine nucleotides of the foot muscle of ark-shell caused by slow freezing

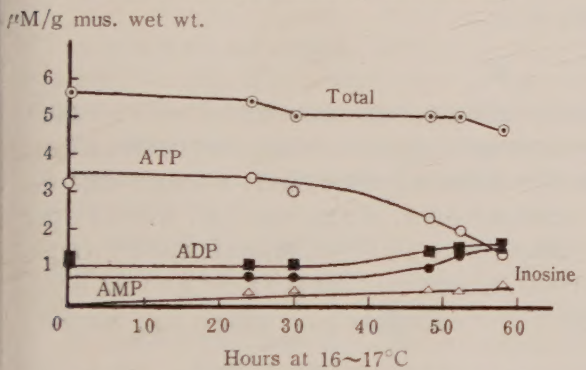


Fig. 4. Changes in adenine nucleotides of the foot muscle of ark-shell at room temperature

のような分解生成物は全く見出されず，この点甚だ特異的であつた。ＡＴＰは凍結貯蔵が３日後で46%，６日後で20%に減少し，ホタテ貝の筋肉の場合と同じ分解速度を示した。

次に筋肉の放置温度が 16～17°Cの 場合の同成分の変化を検討して，第４図のような結果を得た。第４図によると，新鮮筋肉中に存在したATP，ADP，ＡＭＰは，約20時間以上に至るまで殆んど変化せず，30時間以上に及ぶとATPの分解が顕著にすすみ，ＡＤＰ次いでＡＭＰの生成が対応的に見られる。なお，少量の Inosine と推定される物質が20時間以上になると生成してくるが，以後52時間以上に至るまで，顕著な増加を示さず，関連酵素系の活性の低さを暗示するもののようである。なおこのとき，酸可溶核酸成分の総量が減少する傾向が見られるが，この事実は先に述べたホタテ貝及びウバガイばかりでなく，アワビ及びアカザラ[3] などについても同じく観察されることであつて，その原因については現在検討中であるので次号にゆずる。

アカガイ新鮮筋肉中のATP量は，上述の代表的な２例において,3.20～3.67 μ mole/g muscle wet wt., ADP, ＡＭＰはそれぞれ 0.97～1.27, 0.35～1.21μ mole/g muscle wet wt. の値を示し，ウバガイ筋肉の値と非常に近似している。

B) アワビ

アワビは可食肉質部の中，大部分を占める外観上均質の部分について，分析を行つた。

まず，- 5°C附近で筋肉を凍結貯蔵した場合の変化を第５図に示した。第５図によれば，新鮮なアワビ筋肉に主として存在するATPは，凍結貯蔵が25日後に至る間に，非常に緩慢に分解し，対応的にADPが15日後頃を最高に，次いでＡＭＰが一方的に生成，蓄積することがわかつた。ＡＭＰはアカガイにおける場合と同じく，それ以上分解，変化せず Adenosine, Adenine, Inosine, Hypoxanthine のような予想し得る分解生成物は見出すことが出来なかつた。この点アカガイ筋肉と同じく甚だ特異的である。ATPは凍結貯蔵が５日後で65%，15日後で21%まで分解しているが，この分解速度は現在まで検討したホタテ貝，ウバガイ，アカガイ，アカザラ[3] などに比べて，非常に遅いものである。

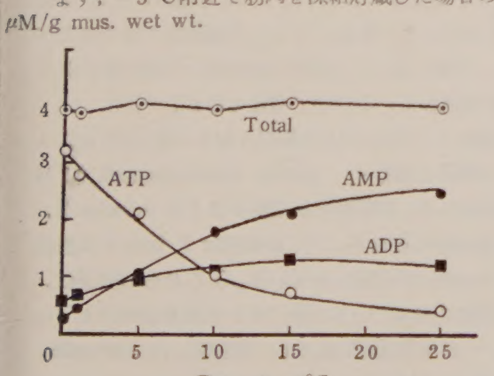


Fig. 5. Changes in adenine nucleotides of the foot muscle of abalone caused by slow freezing

次に筋肉の放置温度が 15～16°C の場合の変化を検

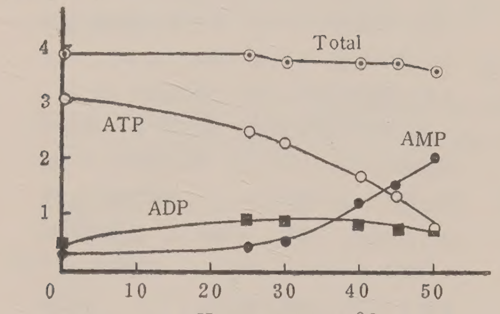


Fig. 6. Changes in adenine nucleotides of the foot muscle of abalone at room temperature

討して，その結果を第6図に示した。第6図によると新鮮筋肉中の主成分を成しているATPをはじめ，ADP，AMPの各成分は，約20時間に至るまで殆んど変化しないが，25時間後に至るとATPの緩慢な分解が起り，ADPは殆んど顕著な増減を示さないが，30時間以後になるとAMPの生成が顕著となつてくる。またこの場合も，AMPの分解生成物は全く見出すことが出来ず関連酵素系の欠除を暗示しているようである。

新鮮アワビ筋肉中のATP，ADP，AMPは，上記の2例においてそれぞれ 2.40〜3.08，0.38〜0.50，0.15〜0.25 μ mole/g muscle wet wt. であり，ホタテ貝，ウバガイ及びアカガイなどに比べて少ない値であつた。

考　　察

筋肉中に存在するATPを中心とする酸可溶核酸成分の絶対量及び死後における変化は，いくつかの要因からなる筋肉の生理状態によつて影響をうけるであろう事をすでに述べた[1]。本報告にあげた結果は典型となる2例であつて，人間の肉眼的な観察による判断では予想出来得ない結果の変動は，ある程度不可避のもののようであつた。即ちアワビ筋肉を -5°C 附近で凍結貯蔵する場合，第3図においては，ATPは5日後まで65%分解をうけるにすぎないが，他の実験では32%まで分解をうけていた。対応的にADP及びAMPが最大生成を示すに至る時間は，第3図の15，25日に対し，他の実験では5,15日後であつて速やかであつた。これらの個体による変化速度のずれは，著者が経験したすべての動物筋肉において観察されたが，各成分の変化の相対的な関連については変りはなかつた。

新鮮筋肉中に見出されるATPの全酸可溶核酸成分量に対する割合は，本報告におけるアカガイでは 60〜73%，アワビでは80〜85%であつて，すでに報じたイカ，ホタテ貝，ウバガイなどの筋肉の値と比べると，アカガイの場合は特に少ない。これらの値もまた，上述したようないくつかの要因に支配されるものと考えている。

アカガイ及びアワビ両筋肉を 16〜17°C で貯蔵するとき，筋肉中のATPが約20時間後に至る間分解をうけず，AMP及びADPも変化しない現象は，先に報じたホタテ貝及びウバガイについても認められる貝類独特の共通現象であつて，特にアカガイ筋肉の場合，筋肉を切りとつた直後よりも1日経過後の場合の方がかえつてATPが多くADP，AMPが減少していることは，室温においてATPの再生産が，筋肉を切りとつた後もかなり長時間にわたつて，行われている可能性を暗示しているものと考えている。

アカガイ及びアワビ両筋肉におけるATPの酵素分解生成物としては，ADP及びAMPが認められ，AMPは最終生成物のように蓄積し，ホタテ貝及びウバガイの場合と同じく，Inosine monophosphate（IMP）は全く生成しなかつた。このときアカガイの筋肉の場合にのみ，貯蔵温度が低温のときは Adenine が，高温のときは Inosine と推定される物質が微量生成する事実が認められた。（この事実はアカガイ貝柱筋肉[4]で特に著るしく，斧足筋の場合に比べると，多量の Adenine が確認されている。）これらの結果は，著者が先に報じたホタテ貝及びウバガイの場合と比べて，非常に異つている。更にホタテ貝及びウバガイの実験例[1]と同じく，アカガイ及びアワビ両筋肉ホモジェネートを粗酵素液として使用し，Adenosine，AMP，IMP等の基質に対する酵素活性の有無を検討した結果，AMP-deaminase及びAdenosine-deaminaseは全く認められず，非常に特異的であつた。またAMPよりは Adenosine を基質とした場合に，より多くの

Adenine を生成する酵素活性（Riboside hydrolase[5] または Nucleoside phosphorylase[6]）が，両筋肉ホモジェネートについて認められた[7]。この酵素反応は 20〜30°C の温度で，10〜300 分の反応時間にわたつて検討されたにも拘らず，両筋肉ホモジェネートの場合共，反応生成物としての Inosine は認められることはなかつたから，特にアカガイの筋肉で見出された Inosine と推定される物質は，筋肉内の酵素の作用で生成したものかどうか疑わしいと考えている。

以上述べたような結果から，アカガイ及びアワビ両筋肉におけるATPの酵素分解の主径路は，ATP→ADP→AMP→Adenosine→Adenine であつて，先に報じたホタテ貝，ウバガイ及びイカ[8] の場合の主径路 ATP→ATP→AMP→Adenosine→Inosine→Hypoxanthine または魚類[9][10][2] 及び哺乳類[11][12] の場合の主径路，ATP→ADP→AMP→IMP→Inosine→Hypoxanthine と明らかに異つていると考えられる。この事実は，比較生化学的見地から甚だ興味深い問題である。

要　　約

ホタテ貝及びウバガイ両筋肉を -5°C または 20°C において貯蔵する間に，筋肉中の酸可溶核酸成分に起る変化について先に報じた。本報告ではアカガイ及びアワビ両筋肉に起る同様条件下での変化について述べる。その結果は次のように総括出来る。

1）新鮮なアカガイ及びアワビ両筋肉中の酸可溶核酸成分は，ATP, ADP, AMPがそれぞれ3.20〜3.67, 0.97〜1.27, 0.35〜1.21 及び 2.40〜3.08, 0.38〜0.50, 0.15〜0.25 μ mole/g muscle wet wt. であつて，ATPが主成分であつた。

2）両筋肉を -5° Cで凍結貯蔵すると，ATPは分解し，ADP次いでAMP が生成し蓄積する。アカガイ筋肉の場合にのみ微量の Adenine が見出されたが，このAMPはそれ以上変化せず，ホタテガイ及びウバガイの場合と非常に異つている。一般的にアカガイ筋肉の方が変化が速やかであつた。

3）両筋肉を室温に放置すると，初めから20時間に至るまで殆んど変化が起らず，ATPは高いレベルに保たれるが，以後緩慢な分解が起りADP次いでAMPが生成する。アカガイ筋肉の場合のみ微量のInosineと推定される物質が見出された。

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